```
=> e kim bum joon/au
E1            6     KIM BUM J/AU
E2           30     KIM BUM JIN/AU
E3          144 --> KIM BUM JOON/AU
E4           19     KIM BUM JUN/AU
E5            1     KIM BUM JUNE/AU
E6            7     KIM BUM KI/AU
E7            1     KIM BUM KWAN/AU
E8            4     KIM BUM KWON/AU
E9            3     KIM BUM KYENG/AU
E10           1     KIM BUM KYEONG/AU
E11           1     KIM BUM KYU/AU
E12           4     KIM BUM MAN/AU

=> s e1-e5 and mycobacter? and (hsp 65)
L1            4 ("KIM BUM J"/AU OR "KIM BUM JIN"/AU OR "KIM BUM JOON"/AU OR
                "KIM BUM JUN"/AU OR "KIM BUM JUNE"/AU) AND MYCOBACTER? AND (HSP
               65)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2            4 DUP REM L1 (0 DUPLICATES REMOVED)

=> s e1-e5 and mycobacter? and (hsp 65)
L3            4 ("KIM BUM J"/AU OR "KIM BUM JIN"/AU OR "KIM BUM JOON"/AU OR
                "KIM BUM JUN"/AU OR "KIM BUM JUNE"/AU) AND MYCOBACTER? AND (HSP
               65)

=> s e1-e5 and mycobacter? and primer?
L4           12 ("KIM BUM J"/AU OR "KIM BUM JIN"/AU OR "KIM BUM JOON"/AU OR
                "KIM BUM JUN"/AU OR "KIM BUM JUNE"/AU) AND MYCOBACTER? AND PRIME
               R?

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5           10 DUP REM L4 (2 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 10 ANSWERS - CONTINUE? Y/(N):y

L5   ANSWER 1 OF 10  USPATFULL on STN
AN     2005:324251  USPATFULL
TI     Rpob gene of streptomyces, primer specific to streptomyces,
       and identification method of streptomyces having rifampin resistance or
       sensitivity by using the same
IN     Kim, Bum-Joon, Jeju-city, KOREA, REPUBLIC OF
       Cho, Moo-Jae, Jeju-city, KOREA, REPUBLIC OF
       Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
       Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
       Park, Jung-Min, Seoul, KOREA, REPUBLIC OF
       Kim, Chang-Jin, Daejeon, KOREA, REPUBLIC OF
PI     US 2005282159      A1   20051222
AI     US 2003-486669     A1   20020711 (10)
       WO 2002-KR1318          20020711
                               20050620  PCT 371 date
PRAI   KR 2001-48983       20010814
       KR 2003-200236731   20020628
       KR 2003-200239464   20020708
DT     Utility
FS     APPLICATION
LREP   KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,
       IRVINE, CA, 92614, US
CLMN   Number of Claims: 20
ECL    Exemplary Claim: 1
```

DRWN 12 Drawing Page(s)
LN.CNT 2729
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a polynucleotide having a 306-bp fragment of an ANA polymerase gene subunit B (rpoB) of Streptomyces, and an identifying method of Streptomyces species using the same. According to the identifying method, the Streptomyces can be detected or identified accurately, economically, and easily. In addition, the identifying method of rifampin-resistant and rifampin-sensitive Streptomyces is a molecular-biological method having advantages in efficiency in terms of cost and time, and accuracy, and which can be widely used for identifying the Streptomyces species in the future.

L5 ANSWER 2 OF 10 USPATFULL on STN
AN 2005:16756 USPATFULL
TI Primers for amplifying hsp 65 gene of mycobacterial species, hsp 65 gene fragments and method of identifying mycobacterial species with the same
IN Kim, Bum-Joon, Jeju-do, KOREA, REPUBLIC OF
Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
PI US 2005014157 A1 20050120
AI US 2004-500586 A1 20040909 (10)
WO 2003-KR131 20030121
PRAI KR 2002-4297 20020124
KR 20020305
DT Utility
FS APPLICATION
LREP ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 2057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

L5 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2005:620828 CAPLUS
DN 144:206426
TI PCR restriction fragment length polymorphism analysis (PRA)-algorithm targeting 644 bp Heat Shock Protein 65 (hsp65) gene for differentiation of Mycobacterium spp.
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park, Young-Gil; Lee, Sueng-Hyun; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon
CS Department of Microbiology and Liver Research Institute, College of Medicine, Seoul National University Chongno-gu, 28 Yongon-dong, Chongno-gu, Seoul, 110-799, S. Korea
SO Journal of Microbiological Methods (2005), 62(2), 199-209
CODEN: JMIMDQ; ISSN: 0167-7012
PB Elsevier B.V.

DT Journal
LA English
AB A method based on PCR-restriction fragment length polymorphism anal. (PRA) using a novel region of the hsp65 gene was developed for the rapid and exact identification of mycobacteria to the species level. A 644 bp region of hsp65 in 62 mycobacteria reference strains, and 4 related bacterial strains was amplified, and the amplified DNAs were subsequently digested with restriction enzymes, namely, AvaII, HphI, and HpaII. Most of the mycobacteria species were easily differentiated at the species level by the developed method. In particular, the method enabled the separation of M. avium, M. intracellulare and M. tuberculosis to the species level by AvaII digestion alone. An algorithm was constructed based on the results and a blind test was successfully performed on 251 clin. isolates, which had been characterized by conventional biochem. testing. Our results suggest that this novel PRA offers a simple, rapid, and accurate method for the identification of mycobacteria culture isolates at the species level.
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 10 USPATFULL on STN
AN 2004:334791 USPATFULL
TI Identification method of genus streptomyces by using groEL2 gene
IN Kim, Bum-Joon, Seoul, KOREA, REPUBLIC OF
Kim, Chang-Jin, Daejeon, KOREA, REPUBLIC OF
Ko, Young Hwan, Jeju-do, KOREA, REPUBLIC OF
Koh, Jeong-Sam, Jeju-do, KOREA, REPUBLIC OF
Park, Dong-Jin, Daejeon, KOREA, REPUBLIC OF
Lee, Hyang Burm, Daejeon, KOREA, REPUBLIC OF
Kim, Hong, Seoul, KOREA, REPUBLIC OF
Kim, Sun-huyn, Seoul, KOREA, REPUBLIC OF
PA KOREA RESEARCH INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY (non-U.S. corporation)
PI US 2004265873 A1 20041230
AI US 2004-824527 A1 20040415 (10)
PRAI KR 2003-24656 20030418
KR 2003-80580 20031114
DT Utility
FS APPLICATION
LREP Finnegan, Henderson, Farabow,, Garrett & Dunner, L.L.P., 1300 I Street, L.L.P., Washington, DC, 20005-3315
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 6 Drawing Page(s)
LN.CNT 1472
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a method for identifying Streptomyces species using groEL2 gene that can compensate for drawbacks of conventional methods of morphologic classification and 16S rDNA identification being time-consuming, unfaithful, and expensive, thus enabling to efficiently identify Streptomyces species.

L5 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
AN 2004:299764 CAPLUS
DN 141:18251
TI Differential identification of Mycobacterium tuberculosis complex and nontuberculous mycobacteria by duplex PCR assay using the RNA polymerase gene (rpoB)
AU Kim, Bum-Joon; Hong, Seong-Karp; Lee, Keun-Hwa; Yun, Yeo-Jun; Kim, Eui-Chong; Park, Young-Gil; Bai, Gil-Han; Kook, Yoon-Hoh
CS Department of Microbiology and Cancer Research Institute, Institute of Endemic Diseases, SNUMRC, Clinical Research Institute, Seoul National University College of Medicine, Seoul, 110-799, S. Korea
SO Journal of Clinical Microbiology (2004), 42(3), 1308-1312

CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB A novel duplex PCR method that can amplify the 235- and 136-bp rpoB DNAs of Mycobacterium tuberculosis complex and nontuberculous mycobacteria (NTM), resp., with two different sets of primers was used to differentially identify 44 reference strains and 379 clin. isolates of mycobacteria in a single-step assay. Showing 100% sensitivity and specificity, the duplex PCR method could clearly differentiate M. tuberculosis complex and NTM strains. In addition, restriction fragment length polymorphism anal. and direct sequencing of the amplicon of NTM could be used to supplement species identification.
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:461814 CAPLUS
DN 141:255044
TI Simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and nontuberculous mycobacteria by polymerase chain reaction-single strand conformation polymorphism and sequence analysis of the RNA polymerase gene (rpoB)
AU Kim, Bum-Joon; Lee, Keun-Hwa; Yun, Yeo-Jun; Park, Eun-Mi; Park, Young-Gil; Bai, Gil-Han; Cha, Chang-Yong; Kook, Yoon-Hoh
CS Seoul National University Hospital, Department of Microbiology and Cancer Research Institute, Institute of Endemic Diseases, SNUMRC, and Clinical Research Institute, Seoul National University College of Medicine, Seoul, 110-799, S. Korea
SO Journal of Microbiological Methods (2004), 58(1), 111-118
CODEN: JMIMDQ; ISSN: 0167-7012
PB Elsevier Science B.V.
DT Journal
LA English
AB Interspecies variations and mutations associated with rifampin resistance in rpoB of Mycobacterium allow for the simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and non-tuberculous mycobacteria by PCR-SSCP anal. and PCR-sequencing. One hundred and ten strains of rifampin-susceptible M. tuberculosis, 14 strains of rifampin-resistant M. tuberculosis, and four strains of the M. avium complex were easily identified by PCR-SSCP. Of another seven strains, which showed unique SSCP patterns, three were identified as rifampin-resistant M. tuberculosis and four as M. terrae complex by subsequent sequence anal. of their rpoB DNAs (306 bp). These results were concordant with those obtained by susceptibility testing, biochem. identification, and 16S rDNA sequencing.
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2003:591378 CAPLUS
DN 139:146183
TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species
IN Kim, Bum-joon; Kook, Yoon-ho; Kim, Jeong-mi
PA Biomedlab Corporation, S. Korea
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 2003062470 A1 20030731 WO 2003-KR131 20030121
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
KR 2003063935 A 20030731 KR 2002-4297 20020124
KR 2003072087 A 20030913 KR 2002-11648 20020305
US 2005014157 A1 20050120 US 2004-500586 20040909
PRAI KR 2002-4297 A 20020124
KR 2002-11648 A 20020305
WO 2003-KR131 W 20030121

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 10 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 2
AN 2001:539286 BIOSIS
DN PREV200100539286
TI Method for identifying mycobacterial species by comparative sequence analysis of rpoB gene.
AU Kook, Yoon-Hoh [Inventor, Reprint author]; Kim, Bum-Joon [Inventor]
CS Seoul, South Korea
ASSIGNEE: Bioneer Corporation, Chooncheongbuk-Do, South Korea
PI US 6242584 20010605
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 5, 2001) Vol. 1247, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The present invention relates to a method for detecting and identifying mycobacterial species which comprises steps of amplifying 342 bp of rpoB gene fragments from clinically isolated mycobacterial using mycobacterial rpoB-specific PCR primers; sequencing 306 bp regions of the amplified 342 bp of rpoB gene fragments except the primer regions; and, inferring a phylogenetic tree with reference species. In accordance with the present invention, it was found that rpoB sequences from 44 mycobacterial species provide a basis for systematic phylogenetic relationship which can be used to identify clinically isolated mycobacteria that are pathogenic or potentially so. Accordingly, the amplification of rpoB DNA followed by automated sequencing and the analysis of phylogenetic relationships with the reference species can be used efficiently to detect and identify clinical isolates of mycobacteria which have not been identified

by the conventional methods. In particular, this approach is useful for slowly growing, fastidious or uncultivable mycobacteria. Furthermore, in the case of M. tuberculosis, rifampin susceptibility can be simultaneously determined. Thus, the PCR- mediated comparative sequence analysis of rpoB DNA of the invention can be regarded as a reliable and rapid method for the diagnosis of mycobacterial infection.

L5 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1999:96395 CAPLUS
DN 130:163954
TI Detection and diagnostic identification of Mycobacterium by amplification of the rpoB gene by nested PCR and sequencing of the products
IN Kook, Yoon-hoh; Kim, Bum-joon
PA Bioneer Corporation, S. Korea
SO PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9905316	A1	19990204	WO 1998-KR228	19980728
	W: AU, CA, CN, JP, RU, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	KR 234975	B1	19991215	KR 1997-35501	19970728
	AU 9884648	A1	19990216	AU 1998-84648	19980728
	US 6242584	B1	20010605	US 1999-147935	19990319
PRAI	KR 1997-35501	A	19970728		
	WO 1998-KR228	W	19980728		

AB A method for detecting and identifying mycobacterial species by nested PCR of a 342 bp fragment of the rpoB gene from clin. isolates followed by sequencing of the 306 bp internal amplification product is described. The phylogenetic position of the isolate can be inferred by comparison with sequences from reference organisms. It was found that rpoB sequences from 44 mycobacterial species provide a basis for systematic phylogenetic relationship which can be used to identify clin. isolated mycobacteria that are pathogenic or potentially so. Amplification of rpoB DNA followed by automated sequencing and the anal. of phylogenetic relationships with the reference species can be used efficiently to detect and identify clin. isolates of mycobacteria which have not been identified by the conventional methods. In particular, this approach is useful for slowly growing, fastidious or uncultivable mycobacteria. Furthermore, in the case of M. tuberculosis, rifampin susceptibility can be simultaneously determined Thus, the PCR-mediated comparative sequence anal. of rpoB DNA of the invention can be regarded as a reliable and rapid method for the diagnosis of mycobacterial infection.
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1997:87310 CAPLUS
DN 126:140284
TI Mutations in the rpoB gene of Mycobacterium tuberculosis that interfere with PCR-single-strand conformation polymorphism analysis for rifampin susceptibility testing
AU Kim, Bum-Joon; Kim, Seok-Yong; Park, Byoung-Hee; Lyu, Mi-Ae; Park, Il-Kyoo; Bai, Gill-Han; Kim, Sang-Jae; Cha, Chang-Yong; Kook, Yoon-Hoh
CS Department of Microbiology and Cancer Research Center, Seoul National University College of Medicine, Seoul, S. Korea

SO Journal of Clinical Microbiology (1997), 35(2), 492-494
CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB Rifampin susceptibility of 32 rifampin-resistant and 26 rifampin-susceptible Mycobacterium tuberculosis strains was analyzed by PCR-single-strand conformation polymorphism (SSCP) and DNA sequencing within the 157-bp region of the rpoB gene (Ala500 to Val550). Two false-pos. PCR-SSCP results were observed among the susceptible strains due to the silent mutation Gln513 (CAA→CAG) and the deletion mutation Thr508 and Ser509. Another silent mutation [Leu511 (CTG→CTA)], combined with the mutation Ser531→Leu, was observed in a resistant strain. These results suggest that to rule out false-pos. PCR-SSCP results, sequencing of the target DNA is required.
RE.CNT 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l2 bib ab 1-
YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 4 USPATFULL on STN
AN 2005:16756 USPATFULL
TI Primers for amplifying hsp 65 gene of mycobacterial species, hsp 65 gene fragments and method of identifying mycobacterial species with the same
IN Kim, Bum-Joon, Jeju-do, KOREA, REPUBLIC OF
Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
PI US 2005014157 A1 20050120
AI US 2004-500586 A1 20040909 (10)
WO 2003-KR131 20030121
PRAI KR 2002-4297 20020124
KR 20020305
DT Utility
FS APPLICATION
LREP ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 2057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

L2 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2005:889455 CAPLUS
DN 144:167106
TI Differentiation of Mycobacterium species by analysis of the heat-shock protein 65 gene (hsp65)
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park,

Young-Gil; Lee, Sueng-Hyun; Chae, Gue-Tae; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon
CS Department of Microbiology, College of Medicine, Seoul National University, Seoul, 110-799, S. Korea
SO International Journal of Systematic and Evolutionary Microbiology (2005), 55(4), 1649-1656
CODEN: ISEMF5; ISSN: 1466-5026
PB Society for General Microbiology
DT Journal
LA English
AB The nucleotide sequences (604 bp) of partial heat-shock protein genes (hsp65) from 161 Mycobacterium strains containing 56 reference Mycobacterium species and 105 clin. isolates were determined and compared. Hsp65 sequence anal. showed a higher degree of divergence between Mycobacterium species than did 16S rRNA gene anal. Generally, the topol. of the phylogenetic tree based on the hsp65 DNA sequences was similar to that of the 16S rRNA gene, thus revealing natural relationships among Mycobacterium species. When a direct sequencing protocol targeting 422 bp sequences was applied to 70 non-tuberculous mycobacterium (NTM) clin. isolates, all NTMs were clearly identified. In addition, an XhoI PCR restriction fragment length polymorphism anal. method for the differentiation of Mycobacterium tuberculosis complex from NTM strains was developed during this study. The results obtained suggest that 604 bp hsp65 sequences are useful for the phylogenetic anal. and species identification of mycobacteria.
RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2005:620828 CAPLUS
DN 144:206426
TI PCR restriction fragment length polymorphism analysis (PRA)-algorithm targeting 644 bp Heat Shock Protein 65 (hsp65) gene for differentiation of Mycobacterium spp.
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park, Young-Gil; Lee, Sueng-Hyun; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon
CS Department of Microbiology and Liver Research Institute, College of Medicine, Seoul National University Chongno-gu, 28 Yongon-dong, Chongno-gu, Seoul, 110-799, S. Korea
SO Journal of Microbiological Methods (2005), 62(2), 199-209
CODEN: JMIMDQ; ISSN: 0167-7012
PB Elsevier B.V.
DT Journal
LA English
AB A method based on PCR-restriction fragment length polymorphism anal. (PRA) using a novel region of the hsp65 gene was developed for the rapid and exact identification of mycobacteria to the species level. A 644 bp region of hsp65 in 62 mycobacteria reference strains, and 4 related bacterial strains was amplified, and the amplified DNAs were subsequently digested with restriction enzymes, namely, AvaII, HphI, and HpaII. Most of the mycobacteria species were easily differentiated at the species level by the developed method. In particular, the method enabled the separation of M. avium, M. intracellulare and M. tuberculosis to the species level by AvaII digestion alone. An algorithm was constructed based on the results and a blind test was successfully performed on 251 clin. isolates, which had been characterized by conventional biochem. testing. Our results suggest that this novel PRA offers a simple, rapid, and accurate method for the identification of mycobacteria culture isolates at the species level.
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2003:591378 CAPLUS
DN 139:146183
TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species
IN Kim, Bum-joon; Kook, Yoon-ho; Kim, Jeong-mi
PA Biomedlab Corporation, S. Korea
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003062470	A1	20030731	WO 2003-KR131	20030121
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	KR 2003063935	A	20030731	KR 2002-4297	20020124
	KR 2003072087	A	20030913	KR 2002-11648	20020305
	US 2005014157	A1	20050120	US 2004-500586	20040909
PRAI	KR 2002-4297	A	20020124		
	KR 2002-11648	A	20020305		
	WO 2003-KR131	W	20030121		

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> e kook yoon ho/au

E1	2		KOOK YOON BUM/AU
E2	1		KOOK YOON HAWN/AU
E3	5	-->	KOOK YOON HO/AU
E4	172		KOOK YOON HOH/AU
E5	1		KOOK YOON HOH*/AU
E6	10		KOOK YOON HWAN/AU
E7	4		KOOK YOON SANG/AU
E8	1		KOOK YOONAH/AU
E9	1		KOOK YOONBUM/AU
E10	1		KOOK YOONHO/AU
E11	14		KOOK YOONHOH/AU
E12	6		KOOK YOUN JAE/AU

=> s e3-e5 and mycobact? and (primer? or HSP?)

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L6          17 ("KOOK YOON HO"/AU OR "KOOK YOON HOH"/AU OR "KOOK YOON HOH*"/AU)
               AND MYCOBACT? AND (PRIMER? OR HSP?)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           11 DUP REM L6 (6 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L7   ANSWER 1 OF 11  USPATFULL on STN
AN     2005:324251  USPATFULL
TI     Rpob gene of streptomyces, primer specific to streptomyces,
       and identification method of streptomyces having rifampin resistance or
       sensitivity by using the same
IN     Kim, Bum-Joon, Jeju-city, KOREA, REPUBLIC OF
       Cho, Moo-Jae, Jeju-city, KOREA, REPUBLIC OF
         Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
       Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
       Park, Jung-Min, Seoul, KOREA, REPUBLIC OF
       Kim, Chang-Jin, Daejeon, KOREA, REPUBLIC OF
PI     US 2005282159      A1   20051222
AI     US 2003-486669     A1   20020711 (10)
       WO 2002-KR1318          20020711
                               20050620  PCT 371 date
PRAI   KR 2001-48983       20010814
       KR 2003-200236731   20020628
       KR 2003-200239464   20020708
DT     Utility
FS     APPLICATION
LREP   KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,
       IRVINE, CA, 92614, US
CLMN   Number of Claims: 20
ECL    Exemplary Claim: 1
DRWN   12 Drawing Page(s)
LN.CNT 2729
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention relates to a polynucleotide having a 306-bp
       fragment of an ANA polymerase gene subunit B (rpoB) of Streptomyces, and
       an identifying method of Streptomyces species using the same. According
       to the identifying method, the Streptomyces can be detected or
       identified accurately, economically, and easily. In addition, the
       identifying method of rifampin-resistant and rifampin-sensitive
       Streptomyces is a molecular-biological method having advantages in
       efficiency in terms of cost and time, and accuracy, and which can be
       widely used for identifying the Streptomyces species in the future.

L7   ANSWER 2 OF 11  USPATFULL on STN
AN     2005:16756  USPATFULL
TI     Primers for amplifying hsp 65 gene of
       mycobacterial species, hsp 65 gene fragments and
       method of identifying mycobacterial species with the same
IN     Kim, Bum-Joon, Jeju-do, KOREA, REPUBLIC OF
         Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
       Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
PI     US 2005014157      A1   20050120
AI     US 2004-500586     A1   20040909 (10)
       WO 2003-KR131           20030121
PRAI   KR 2002-4297        20020124
       KR                  20020305
DT     Utility
FS     APPLICATION
LREP   ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE
       4000, CHARLOTTE, NC, 28280-4000
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CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 2057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

L7 ANSWER 3 OF 11 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 1
AN 2005:448449 BIOSIS
DN PREV200510237956
TI Differentiation of Mycobacterium species by analysis of the heat-shock protein 65 gene (hsp65).
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park, Young-Gil; Lee, Sueng-Hyun; Chae, Gue-Tae; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon [Reprint Author]
CS Seoul Natl Univ, Coll Med, Dept Microbiol, 28 Yongon Dong, Seoul 110799, South Korea
kbumjoon@snu.ac.kr
SO International Journal of Systematic and Evolutionary Microbiology, (JUL 2005) Vol. 55, No. Part 4, pp. 1649-1656.
ISSN: 1466-5026.
DT Article
LA English
ED Entered STN: 3 Nov 2005
Last Updated on STN: 3 Nov 2005
AB The nucleotide sequences (604 bp) of partial heat-shock protein genes (hsp65) from 161 Mycobacterium strains containing 56 reference Mycobacterium species and 105 clinical isolates were determined and compared. hsp65 sequence analysis showed a higher degree of divergence between Mycobacterium species than did 16S rRNA gene analysis. Generally, the topology of the phylogenetic tree based on the hsp65 DNA sequences was similar to that of the 16S rRNA gene, thus revealing natural relationships among Mycobacterium species. When a direct sequencing protocol targeting 422 bp sequences was applied to 70 non-tuberculous mycobacterium (NTM) clinical isolates, all NTMs were clearly identified. In addition, an XhoI PCR restriction fragment length polymorphism analysis method for the differentiation of Mycobacterium, tuberculosis complex from NTM strains was developed during this study. The results obtained suggest that 604 bp hsp65 sequences are useful for the phylogenetic analysis and species identification of mycobacteria.

L7 ANSWER 4 OF 11 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 2
AN 2005:437869 BIOSIS
DN PREV200510224308
TI PCR restriction fragment length polymorphism analysis (PRA)-algorithm targeting 644 bp Heat Shock Protein 65 (hsp65) gene for differentiation of Mycobacterium spp.
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park,

Young-Gil; Lee, Sueng-Hyun; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon [Reprint Author]

CS Seoul Natl Univ, Coll Med, Dept Microbiol, 28 Yongon Dong, Seoul 110799, South Korea
kbumjoon@snu.ac.kr

SO Journal of Microbiological Methods, (AUG 2005) Vol. 62, No. 2, pp. 199-209.
CODEN: JMIMDQ. ISSN: 0167-7012.

DT Article

LA English

ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005

AB A method based on PCR-restiiction fragment length polymorphism analysis (PRA) using a novel region of the hsp65 gene was developed for the rapid and exact identification of mycobacteria to the species level. A 644 bp region of hsp65 in 62 mycobacteria reference strains, and 4 related bacterial strains were amplified, and the amplified DNAs were subsequently digested with restriction enzymes, namely, AvaII, HphI, and HpaII. Most of the mycobacteria species were easily differentiated at the species level by the developed method. In particular, the method enabled the separation of M avium, M intracellulare and M tuberculosis to the species level by AvaII digestion alone. An algorithm was constructed based on the results and a blind test was successfully performed on 251 clinical isolates, which had been characterized by conventional biochemical testing. Our results suggest that this novel PRA offers a simple, rapid, and accurate method for the identification of mycobacteria culture isolates at the species level.

L7 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3

AN 2004:299764 CAPLUS

DN 141:18251

TI Differential identification of Mycobacterium tuberculosis complex and nontuberculous mycobacteria by duplex PCR assay using the RNA polymerase gene (rpoB)

AU Kim, Bum-Joon; Hong, Seong-Karp; Lee, Keun-Hwa; Yun, Yeo-Jun; Kim, Eui-Chong; Park, Young-Gil; Bai, Gil-Han; Kook, Yoon-Hoh

CS Department of Microbiology and Cancer Research Institute, Institute of Endemic Diseases, SNUMRC, Clinical Research Institute, Seoul National University College of Medicine, Seoul, 110-799, S. Korea

SO Journal of Clinical Microbiology (2004), 42(3), 1308-1312
CODEN: JCMIDW; ISSN: 0095-1137

PB American Society for Microbiology

DT Journal

LA English

AB A novel duplex PCR method that can amplify the 235- and 136-bp rpoB DNAs of Mycobacterium tuberculosis complex and nontuberculous mycobacteria (NTM), resp., with two different sets of primers was used to differentially identify 44 reference strains and 379 clin. isolates of mycobacteria in a single-step assay. Showing 100% sensitivity and specificity, the duplex PCR method could clearly differentiate M. tuberculosis complex and NTM strains. In addition, restriction fragment length polymorphism anal. and direct sequencing of the amplicon of NTM could be used to supplement species identification.

RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

AN 2004:461814 CAPLUS

DN 141:255044

TI Simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and nontuberculous mycobacteria by polymerase chain reaction-single strand conformation polymorphism and sequence analysis of

the RNA polymerase gene (rpoB)
AU Kim, Bum-Joon; Lee, Keun-Hwa; Yun, Yeo-Jun; Park, Eun-Mi; Park, Young-Gil; Bai, Gil-Han; Cha, Chang-Yong; Kook, Yoon-Hoh
CS Seoul National University Hospital, Department of Microbiology and Cancer Research Institute, Institute of Endemic Diseases, SNUMRC, and Clinical Research Institute, Seoul National University College of Medicine, Seoul, 110-799, S. Korea
SO Journal of Microbiological Methods (2004), 58(1), 111-118
CODEN: JMIMDQ; ISSN: 0167-7012
PB Elsevier Science B.V.
DT Journal
LA English
AB Interspecies variations and mutations associated with rifampin resistance in rpoB of Mycobacterium allow for the simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and non-tuberculous mycobacteria by PCR-SSCP anal. and PCR-sequencing. One hundred and ten strains of rifampin-susceptible M. tuberculosis, 14 strains of rifampin-resistant M. tuberculosis, and four strains of the M. avium complex were easily identified by PCR-SSCP. Of another seven strains, which showed unique SSCP patterns, three were identified as rifampin-resistant M. tuberculosis and four as M. terrae complex by subsequent sequence anal. of their rpoB DNAs (306 bp). These results were concordant with those obtained by susceptibility testing, biochem. identification, and 16S rDNA sequencing.
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2003:591378 CAPLUS
DN 139:146183
TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species
IN Kim, Bum-joon; Kook, Yoon-ho; Kim, Jeong-mi
PA Biomedlab Corporation, S. Korea
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003062470	A1	20030731	WO 2003-KR131	20030121
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	KR 2003063935	A	20030731	KR 2002-4297	20020124
	KR 2003072087	A	20030913	KR 2002-11648	20020305
	US 2005014157	A1	20050120	US 2004-500586	20040909
PRAI	KR 2002-4297	A	20020124		
	KR 2002-11648	A	20020305		
	WO 2003-KR131	W	20030121		

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification

methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 11 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 4
AN 2001:539286 BIOSIS
DN PREV200100539286
TI Method for identifying mycobacterial species by comparative sequence analysis of rpoB gene.
AU Kook, Yoon-Hoh [Inventor, Reprint author]; Kim, Bum-Joon [Inventor]
CS Seoul, South Korea
ASSIGNEE: Bioneer Corporation, Chooncheongbuk-Do, South Korea
PI US 6242584 20010605
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 5, 2001) Vol. 1247, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002
AB The present invention relates to a method for detecting and identifying mycobacterial species which comprises steps of amplifying 342 bp of rpoB gene fragments from clinically isolated mycobacterial using mycobacterial rpoB-specific PCR primers; sequencing 306 bp regions of the amplified 342 bp of rpoB gene fragments except the primer regions; and, inferring a phylogenetic tree with reference species. In accordance with the present invention, it was found that rpoB sequences from 44 mycobacterial species provide a basis for systematic phylogenetic relationship which can be used to identify clinically isolated mycobacteria that are pathogenic or potentially so. Accordingly, the amplification of rpoB DNA followed by automated sequencing and the analysis of phylogenetic relationships with the reference species can be used efficiently to detect and identify clinical isolates of mycobacteria which have not been identified by the conventional methods. In particular, this approach is useful for slowly growing, fastidious or uncultivable mycobacteria. Furthermore, in the case of M. tuberculosis, rifampin susceptibility can be simultaneously determined. Thus, the PCR- mediated comparative sequence analysis of rpoB DNA of the invention can be regarded as a reliable and rapid method for the diagnosis of mycobacterial infection.

L7 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2001:781887 CAPLUS
DN 135:283951
TI Detection method of rifampin-resistant Mycobacterium tuberculosis by nested PCR-SSCP and single step nested PCR-SSCP targeting rpoB gene
IN Kook, Yoon Hoh; Kim, Beom Jun; Kim, Sang Jae; Bae, Gil Han
PA S. Korea
SO Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DT Patent
LA Korean
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	KR 2000000849	A	20000115	KR 1998-20720	19980603
PRAI	KR 1998-20720		19980603		

AB A detection method of Rifampin-resistant M. tuberculosis by nested PCR-SSCP and single step nested PCR-SSCP targeting a rpoB DNA fragment of the M. tuberculosis is provided which can prevent a false pos. reaction and can save time and expenses by treating many specimens. The detection method of Rifampin-resistant M. tuberculosis is provided by outer PCR using an outer primer, which amplifies only rpoB DNA fragment (205bp) of the M. tuberculosis specifically without amplifying other bacterial rpoB DNA, nested PCR-SSCP (polymerase chain reaction-single strand conformational polymorphism) using an inner primer, which amplifies only rpoB DNA fragment (157bp) of the M. tuberculosis specifically, and single step nested PCR-SSCP. It takes about four days to detect the Rifampin-resistant M. tuberculosis by this method while it takes about twelve weeks by a conventional susceptibility test through a bacterial culture.

L7 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1999:96395 CAPLUS
DN 130:163954
TI Detection and diagnostic identification of Mycobacterium by amplification of the rpoB gene by nested PCR and sequencing of the products
IN Kook, Yoon-hoh; Kim, Bum-joon
PA Bioneer Corporation, S. Korea
SO PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9905316	A1	19990204	WO 1998-KR228	19980728
	W: AU, CA, CN, JP, RU, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	KR 234975	B1	19991215	KR 1997-35501	19970728
	AU 9884648	A1	19990216	AU 1998-84648	19980728
	US 6242584	B1	20010605	US 1999-147935	19990319
PRAI	KR 1997-35501	A	19970728		
	WO 1998-KR228	W	19980728		

AB A method for detecting and identifying mycobacterial species by nested PCR of a 342 bp fragment of the rpoB gene from clin. isolates followed by sequencing of the 306 bp internal amplification product is described. The phylogenetic position of the isolate can be inferred by comparison with sequences from reference organisms. It was found that rpoB sequences from 44 mycobacterial species provide a basis for systematic phylogenetic relationship which can be used to identify clin. isolated mycobacteria that are pathogenic or potentially so. Amplification of rpoB DNA followed by automated sequencing and the anal. of phylogenetic relationships with the reference species can be used efficiently to detect and identify clin. isolates of mycobacteria which have not been identified by the conventional methods. In particular, this approach is useful for slowly growing, fastidious or uncultivable mycobacteria. Furthermore, in the case of M. tuberculosis, rifampin susceptibility can be simultaneously determined Thus, the PCR-mediated comparative sequence anal. of rpoB DNA of the invention can be regarded as a reliable and rapid method for the diagnosis of mycobacterial infection.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L7   ANSWER 11 OF 11  CAPLUS  COPYRIGHT 2006 ACS on STN
AN   1997:87310  CAPLUS
DN   126:140284
TI   Mutations in the rpoB gene of Mycobacterium tuberculosis that
     interfere with PCR-single-strand conformation polymorphism analysis for
     rifampin susceptibility testing
AU   Kim, Bum-Joon; Kim, Seok-Yong; Park, Byoung-Hee; Lyu, Mi-Ae; Park,
     Il-Kyoo; Bai, Gill-Han; Kim, Sang-Jae; Cha, Chang-Yong; Kook,
     Yoon-Hoh
CS   Department of Microbiology and Cancer Research Center, Seoul National
     University College of Medicine, Seoul, S. Korea
SO   Journal of Clinical Microbiology (1997), 35(2), 492-494
     CODEN: JCMIDW; ISSN: 0095-1137
PB   American Society for Microbiology
DT   Journal
LA   English
AB   Rifampin susceptibility of 32 rifampin-resistant and 26
     rifampin-susceptible Mycobacterium tuberculosis strains was
     analyzed by PCR-single-strand conformation polymorphism (SSCP) and DNA
     sequencing within the 157-bp region of the rpoB gene (Ala500 to Val550).
     Two false-pos. PCR-SSCP results were observed among the susceptible strains
     due to the silent mutation Gln513 (CAA→CAG) and the deletion
     mutation Thr508 and Ser509.  Another silent mutation [Leu511
     (CTG→CTA)], combined with the mutation Ser531→Leu, was observed
     in a resistant strain.  These results suggest that to rule out false-pos.
     PCR-SSCP results, sequencing of the target DNA is required.
RE.CNT  19    THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> e kim jeong mi/au
E1            1     KIM JEONG MANN/AU
E2           43     KIM JEONG MEE/AU
E3           64 --> KIM JEONG MI/AU
E4          233     KIM JEONG MIN/AU
E5            3     KIM JEONG MO/AU
E6            4     KIM JEONG MOG/AU
E7           23     KIM JEONG MOK/AU
E8            1     KIM JEONG MONG/AU
E9            1     KIM JEONG MOO/AU
E10          11     KIM JEONG MOOG/AU
E11           4     KIM JEONG MOOK/AU
E12           1     KIM JEONG MOON/AU

=> s e3 and mycobact? and (primer? or HSP?)
L8            5 "KIM JEONG MI"/AU AND MYCOBACT? AND (PRIMER? OR HSP?)

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            5 DUP REM L8 (0 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y


L9   ANSWER 1 OF 5  USPATFULL on STN
AN     2005:324251  USPATFULL
TI     Rpob gene of streptomyces, primer specific to streptomyces,
       and identification method of streptomyces having rifampin resistance or
       sensitivity by using the same
IN     Kim, Bum-Joon, Jeju-city, KOREA, REPUBLIC OF
       Cho, Moo-Jae, Jeju-city, KOREA, REPUBLIC OF
       Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
         Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
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Park, Jung-Min, Seoul, KOREA, REPUBLIC OF
Kim, Chang-Jin, Daejeon, KOREA, REPUBLIC OF
PI US 2005282159 A1 20051222
AI US 2003-486669 A1 20020711 (10)
WO 2002-KR1318 20020711
20050620 PCT 371 date
PRAI KR 2001-48983 20010814
KR 2003-200236731 20020628
KR 2003-200239464 20020708
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614, US
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 2729
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a polynucleotide having a 306-bp fragment of an ANA polymerase gene subunit B (rpoB) of Streptomyces, and an identifying method of Streptomyces species using the same. According to the identifying method, the Streptomyces can be detected or identified accurately, economically, and easily. In addition, the identifying method of rifampin-resistant and rifampin-sensitive Streptomyces is a molecular-biological method having advantages in efficiency in terms of cost and time, and accuracy, and which can be widely used for identifying the Streptomyces species in the future.

L9 ANSWER 2 OF 5 USPATFULL on STN
AN 2005:16756 USPATFULL
TI Primers for amplifying hsp 65 gene of mycobacterial species, hsp 65 gene fragments and method of identifying mycobacterial species with the same
IN Kim, Bum-Joon, Jeju-do, KOREA, REPUBLIC OF
Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
PI US 2005014157 A1 20050120
AI US 2004-500586 A1 20040909 (10)
WO 2003-KR131 20030121
PRAI KR 2002-4297 20020124
KR 20020305
DT Utility
FS APPLICATION
LREP ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 2057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

L9 ANSWER 3 OF 5 USPATFULL on STN
AN 2004:50804 USPATFULL
TI Diagnosis kit for mycobacterium species indentification and drug-resistance detection and manufacturing method thereof
IN Kim, Hyung-Jung, Gyonggi-do, KOREA, REPUBLIC OF
Kim, Na Young, Seoul, KOREA, REPUBLIC OF
Yoon, Sung Wook, Seoul, KOREA, REPUBLIC OF
Kim, Jeong Mi, Seoul, KOREA, REPUBLIC OF
Park, Mi Sun, Busan, KOREA, REPUBLIC OF
PI US 2004038233 A1 20040226
AI US 2003-297134 A1 20030707 (10)
WO 2001-KR904 20010530
PRAI KR 2000-29369 20000530
DT Utility
FS APPLICATION
LREP Frank Chau, F Chau & Associates, Suite 501, 1900 Hempstead Turnpike, East Meadow, NY, 11554
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 9 Drawing Page(s)
LN.CNT 1586
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to diagnosis kit for Mycobacterium species identification and drug-resistance detection and manufacturing method thereof, which can discriminate a Mycobacterium Tuberculosis rpoB gene point mutation relating to the Mycobacterium species identification and drug-resistance swiftly, exactly and in large quantities using an oligonucleotide chip. The diagnosis kit for Mycobacterium species identification and drug-resistance detection in accordance with the present invention consists of an oligonucleotide chip including a Mycobacterium tuberculosis complex probe, a Mycobacterium species identification probe and a drug-resistance detection probe of a Mycobacterium tuberculosis rpoB gene, and a fluorescent material containing a biotin-binding protein so as to detect hybridization of amplified products of a specimen marked as biotine and the corresponding probe.

L9 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2003:591378 CAPLUS
DN 139:146183
TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species
IN Kim, Bum-joon; Kook, Yoon-ho; Kim, Jeong-mi
PA Biomedlab Corporation, S. Korea
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003062470	A1	20030731	WO 2003-KR131	20030121

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	KR 2003063935	A	20030731	KR 2002-4297	20020124
	KR 2003072087	A	20030913	KR 2002-11648	20020305
	US 2005014157	A1	20050120	US 2004-500586	20040909
PRAI	KR 2002-4297	A	20020124		
	KR 2002-11648	A	20020305		
	WO 2003-KR131	W	20030121		

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2001:886558 CAPLUS
DN 136:32814
TI Diagnosis kit for Mycobacterium species identification and drug-resistance detection using species- and strain-specific hybridization probes
IN Kim, Hyun-jung; Kim, Na-young; Yoon, Sung-wook; Kim, Jeong-mi; Park, Mi-sun
PA Biomedlab Co., Ltd., S. Korea
SO PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001092573	A1	20011206	WO 2001-KR904	20010530
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1290224	A1	20030312	EP 2001-936994	20010530
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2003534810	T2	20031125	JP 2002-500764	20010530
US 2004038233	A1	20040226	US 2003-297134	20030707
PRAI KR 2000-29369	A	20000530		
WO 2001-KR904	W	20010530		

AB The present invention relates to diagnosis kit for Mycobacterium species identification and drug-resistance detection based on hybridization probes specific for the DNA formation factor RP-A and movement protein genes. The method can discriminate a Mycobacterium tuberculosis rpoB gene point mutation relating to the Mycobacterium species identification and drug-resistance swiftly, exactly, and in large quantities using an oligonucleotide chip. The diagnosis kit for Mycobacterium species identification and drug-resistance detection in accordance with the present invention

consists of an oligonucleotide chip including a Mycobacterium tuberculosis Mycobacterium species identification probe and a drug-resistance detection probe of a complex probe, a Mycobacterium tuberculosis rpoB gene, and a fluorescent material containing a biotin-binding protein so as to detect hybridization of amplified products of a specimen marked as biotin and the corresponding probe.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s mycobact? and (HSP 65) and primer?
L10 63 MYCOBACT? AND (HSP 65) AND PRIMER?

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11 59 DUP REM L10 (4 DUPLICATES REMOVED)

=> s l11 and ((primer?)(2w)(HSP?))
L12 5 L11 AND ((PRIMER?)(2W)(HSP?))

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L12 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2005:620828 CAPLUS
DN 144:206426
TI PCR restriction fragment length polymorphism analysis (PRA)-algorithm targeting 644 bp Heat Shock Protein 65 (hsp65) gene for differentiation of Mycobacterium spp.
AU Kim, Hong; Kim, Sun-Hyun; Shim, Tae-Sun; Kim, Mi-na; Bai, Gill-Han; Park, Young-Gil; Lee, Sueng-Hyun; Cha, Chang-Yong; Kook, Yoon-Hoh; Kim, Bum-Joon
CS Department of Microbiology and Liver Research Institute, College of Medicine, Seoul National University Chongno-gu, 28 Yongon-dong, Chongno-gu, Seoul, 110-799, S. Korea
SO Journal of Microbiological Methods (2005), 62(2), 199-209
CODEN: JMIMDQ; ISSN: 0167-7012
PB Elsevier B.V.
DT Journal
LA English
AB A method based on PCR-restriction fragment length polymorphism anal. (PRA) using a novel region of the hsp65 gene was developed for the rapid and exact identification of mycobacteria to the species level. A 644 bp region of hsp65 in 62 mycobacteria reference strains, and 4 related bacterial strains was amplified, and the amplified DNAs were subsequently digested with restriction enzymes, namely, AvaII, HphI, and HpaII. Most of the mycobacteria species were easily differentiated at the species level by the developed method. In particular, the method enabled the separation of M. avium, M. intracellulare and M. tuberculosis to the species level by AvaII digestion alone. An algorithm was constructed based on the results and a blind test was successfully performed on 251 clin. isolates, which had been characterized by conventional biochem. testing. Our results suggest that this novel PRA offers a simple, rapid, and accurate method for the identification of mycobacteria culture isolates at the species level.
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI PCR restriction fragment length polymorphism analysis (PRA)-algorithm targeting 644 bp Heat Shock Protein 65 (hsp65) gene for differentiation of Mycobacterium spp.
AB A method based on PCR-restriction fragment length polymorphism anal. (PRA) using a novel region of the hsp65 gene was developed for the rapid and exact identification of mycobacteria to the species level. A 644 bp region of hsp65 in 62 mycobacteria reference strains, and 4 related bacterial strains was amplified, and the amplified DNAs were

subsequently digested with restriction enzymes, namely, AvaII, HphI, and HpaII. Most of the mycobacteria species were easily differentiated at the species level by the developed method. In particular, the method enabled the separation of M. avium, M. intracellulare and M. tuberculosis to the species level by AvaII digestion alone. An algorithm was constructed based on the results and a blind test was successfully performed on 251 clin. isolates, which had been characterized by conventional biochem. testing. Our results suggest that this novel PRA offers a simple, rapid, and accurate method for the identification of mycobacteria culture isolates at the species level.

ST PCR RFLP algorithm hsp65 gene restriction endonuclease Mycobacterium

IT Heat-shock proteins
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(HSP 65; PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

IT Algorithm
Human
Mycobacterium
Mycobacterium avium
Mycobacterium intracellulare
Mycobacterium tuberculosis
PCR (polymerase chain reaction)
RFLP (restriction fragment length polymorphism)
(PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

IT Primers (nucleic acid)
RL: ARG (Analytical reagent use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(PCR; PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

IT 81295-07-0, Restriction endonuclease AvaII 81295-25-2, Restriction endonuclease HpaII 81295-26-3, Restriction endonuclease HphI
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); BIOL (Biological study); USES (Uses)
(PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

IT 875804-98-1
RL: ARG (Analytical reagent use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(PCR primer HSPF3; PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

IT 875804-99-2
RL: ARG (Analytical reagent use); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(PCR primer HSPR4; PCR RFLP algorithm, targeting hsp65 gene, for identification of Mycobacterium in clin. isolates)

L12 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2003:591378 CAPLUS
DN 139:146183
TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species
IN Kim, Bum-joon; Kook, Yoon-ho; Kim, Jeong-mi
PA Biomedlab Corporation, S. Korea
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003062470	A1	20030731	WO 2003-KR131	20030121
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	KR 2003063935	A	20030731	KR 2002-4297	20020124
	KR 2003072087	A	20030913	KR 2002-11648	20020305
	US 2005014157	A1	20050120	US 2004-500586	20040909
PRAI	KR 2002-4297	A	20020124		
	KR 2002-11648	A	20020305		
	WO 2003-KR131	W	20030121		

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species

AB The present invention relates to a pair of primers specific to mycobacterial species, a polynucleotide of an HSP 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp HSP 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence anal. method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on biochem. characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.

ST primer mycobacteria heat shock protein hsp65 gene

IT Nucleic acid amplification (method)
(DNA; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT Heat-shock proteins
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(HSP 65; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT Gene, microbial

RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(HSP 65; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT Diagnosis
(mol.; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT DNA sequences
Mycobacterium
Mycobacterium BCG
Mycobacterium abscessus
Mycobacterium africanum
Mycobacterium aichiense
Mycobacterium asiaticum
Mycobacterium avium
Mycobacterium avium paratuberculosis
Mycobacterium bovis
Mycobacterium celatum
Mycobacterium chelonae
Mycobacterium chitae
Mycobacterium farcinogenes
Mycobacterium flavescens
Mycobacterium fortuitum
Mycobacterium gastri
Mycobacterium genavense
Mycobacterium gordonae
Mycobacterium haemophilum
Mycobacterium interjectum
Mycobacterium intracellulare
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium microti
Mycobacterium mucogenicum
Mycobacterium neoaurum
Mycobacterium nonchromogenicum
Mycobacterium parafortuitum
Mycobacterium peregrinum
Mycobacterium phlei
Mycobacterium scrofulaceum
Mycobacterium senegalense
Mycobacterium shimoidei
Mycobacterium simiae
Mycobacterium smegmatis
Mycobacterium szulgai
Mycobacterium terrae
Mycobacterium thermoresistibile
Mycobacterium triviale
Mycobacterium tuberculosis
Mycobacterium ulcerans
Mycobacterium vaccae
Mycobacterium wolinskyi
Nocardia carnea
RFLP (restriction fragment length polymorphism)
Tsukamurella paurometabola
Tsukamurella pulmonis
Tsukamurella tyrosinosolvens
(primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT Primers (nucleic acid)
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT 569430-56-4 569432-08-2 569432-09-3 569432-10-6 569432-11-7
569432-12-8 569432-13-9 569432-14-0 569432-15-1 569432-16-2
569432-17-3 569432-18-4 569432-19-5 569432-20-8 569432-21-9
569432-22-0 569432-23-1 569432-24-2 569432-25-3 569432-26-4
569432-27-5 569432-28-6 569432-29-7 569432-30-0 569432-31-1
569432-32-2 569432-33-3 569432-34-4 569432-35-5 569432-36-6
569432-37-7 569432-38-8 569432-39-9 569432-40-2 569432-41-3
569432-42-4 569432-43-5 569432-44-6 569432-45-7 569432-46-8
569432-47-9 569432-48-0 569432-49-1 569432-50-4 569432-51-5
569432-52-6 569432-53-7 569432-54-8 569432-55-9
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(nucleotide sequence; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT 569432-56-0
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(primer HSPF3 sequence; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT 569432-57-1
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(primer HSPR3 sequence; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT 81295-43-4, Nuclease, restriction endodeoxyribo-, Xho I
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

IT 569477-29-8
RL: PRP (Properties)
(unclaimed sequence; primers for amplifying mycobacterial heat shock protein HSP 65 gene and use for identifying mycobacterial species)

L12 ANSWER 3 OF 5 USPATFULL on STN
AN 2005:16756 USPATFULL
TI Primers for amplifying hsp 65 gene of mycobacterial species, hsp 65 gene fragments and method of identifying mycobacterial species with the same
IN Kim, Bum-Joon, Jeju-do, KOREA, REPUBLIC OF
Kook, Yoon-Ho, Seoul, KOREA, REPUBLIC OF
Kim, Jeong-Mi, Seoul, KOREA, REPUBLIC OF
PI US 2005014157 A1 20050120
AI US 2004-500586 A1 20040909 (10)
WO 2003-KR131 20030121
PRAI KR 2002-4297 20020124

KR 20020305
DT Utility
FS APPLICATION
LREP ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 2057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.
TI Primers for amplifying hsp 65 gene of mycobacterial species, hsp 65 gene fragments and method of identifying mycobacterial species with the same
AB The present invention relates to a pair of primers specific to mycobacterial spedes, a polynucleotide of an hsp 65 gene fragment, and a method for the identification of mycobacterial species by using the same. More specifically, the 604-bp hsp 65 gene fragment can be applied to identification methods of mycobacteria such as the comparative sequence analysis method, the probe hybridization method, and PCR-RFLP, which can resolve the problems of a conventional identification method based on bio-chemical characteristics, where the genus mycobacterium covers various species and has a low growth rate, and of the problems of 16s rDNA. Thus, according to the identification method of the present invention, the mycobacterial species can be identified simply, economically, and accurately.
SUMM [0003] The present invention relates to a pair of primers specific to Mycobacterial species, more specifically to a pair of primers that can specifically amplify the hsp 65 gene of mycobacteria, a gene fragment of hsp 65, and an identifying method of Mycobacterial species.
SUMM [0005] The genus Mycobacterium covers a wide range of organisms including obligate species causing serious human and animal disease such as tuberculosis, bovine tuberculosis,. . . and saprothytic species found in the natural environment. At present, it is known that about 72 species of the genus mycobacterium have been reported, of which about 25 species are involved in the human diseases.
SUMM [0006] Tuberculosis is the largest of the Mycobacterial infections. The Mycobacterial species causing tuberculosis include M. tuberculosis, M bovis, M. africanum, and M. microti, which are classified as M. tuberculosis complex. . . use of antituberculosis drugs until the end of the 1980s, but in line with the rapid increase of AIDS and Mycobacterium tuberculosis with drug resistance, tuberculosis increased in developed countries in the 1990s. In particular, it has been reported that the. . .
SUMM [0007] Mycobacteria Other than Mycobacterium tuberculosis (MOTT, or nontuberculous mycobacteria, NTM) causes infection in aged people and immuno-compromised patients, and its clinical manifestation is similar to tuberculosis. The occurrence of. .

. infection. It has been reported that MOTT also causes disease in patients who are not immuno-compromised, and that 50% of Mycobacterial infection in the United States is tuberculosis and 50% is MOTT infection over the past 10 years. With the spread. . .

SUMM [0008] Mycobacterial species have different patterns of resistance to antituberculosis drugs from each other, and thus they are treated by different methods with different drugs (Wolinsky E: Mycobacterial diseases other than tuberculosis. Clin Infect Dis 15: 1-10, 1992). Accordingly, Mycobacteria need to be differentiated and identified on a species level.

SUMM [0009] A biochemical method for identifying Mycobacterial species is laborious and time-consuming due to the slow growing rate of Mycobacteria. A cell wall lipid analyzing method using High-performance Uipid Chromatography (HPLC) and Thin Layer Lipid Chromatography`(TLC) is difficult to. . . disadvantage in that it takes a great deal of time to perform due to the slow growing rate of the Mycobacteria (about 2-3 months for slow-growing mycobacteria). Thus, the treatment of Mycobacterial infection can be delayed (Nolte F S, Metchock B: Mycobacterium , In Murray P R, Baron E J, Pfaller M A, Tenover F C, Yolken R H (eds.), Manual of clinical. . .

SUMM [0010] 16s rDNA is commonly used as a chronometer molecule for identification of the Mycobacterial species with a molecular biological method. In 1990, the nucleic acid sequence of 16s rDNA was analyzed, and it shows the phylogenetic relationship of Mycobacteria well. Until now, various methods of identifying Mycobacterial species by using the 16S rDNA have been developed and studied (Comparative sequence analysis, Probe hybridization, and Polymerization chain reaction-restriction. . .

SUMM [0011] Identifying methods of Mycobacterial species by using dnaJ and 23S rDNA as alternative chronometers were developed in 1994. However, dnaJ and 23S rDNA have. . . A M, Van Schalkwyk E J, Coetzee G J, Van Helden P D. Strain-specific variation in the dnaJ gene of mycobacteria. J Med Microbiol. 44(5):332-339, 1996). In 1993, Telenti A et al. reported that a method for the identification of mycobacteria at the species level was developed by using polymerase chain reaction (PCR)-Restriction Enzyme Length Polymorphism of a gene fragment of hsp 65. The method involves steps of amplifying an hsp 65 gene fragment by PCR and restriction enzyme analysis of PCR products of hsp 65 with two restriction enzymes, BstEII and HaeIII, and 29 species and subspecies were differentiated by PCR-restriction enzyme pattern analysis. (Telenti A, Marchesi F, Balz M, Bally F, Bottger E C, Bodmer T. "Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis," J. Clin. Microbiol. 31 (2):175-8. 1993).

SUMM [0012] However, the above methods for identifying mycobacteria are disadvantageous in that the procedure involves various restriction enzymes and is expensive. In addition, the gene fragment must be. . . 10 bp fragment due to the small size of the restriction enzyme fragment in the case of Hae III. Also, mycobacterial species must be identified accurately on the basis of a known restriction fragment database of each species, or they must. . .

SUMM [0013] To resolve the above problems, an object of the present invention is to provide a pair of primers for amplifying the hsp65 gene of mycobacteria.

SUMM . . . object of the present invention is to provide a polynucleotide of the hsp65 gene fragment that is amplified with the primers.

SUMM . . . is yet another object of the present invention to provide a probe or a probe set for detecting or identifying mycobacterial species comprising at least a gene fragment of the hsp 65 gene of reference mycobacterial species.

SUMM . . . still another object of the present invention to provide a simple and accurate method for the detection or identification of

mycobacterial species.

SUMM [0017] It is a further object of the present invention to provide a method for the identification of mycobacterial species comprising the steps of:

SUMM [0018] (1) amplifying an hsp 65 gene fragment of mycobacteria of interest with primers for specifically amplifying the hsp 65 gene of mycobacteria ;

SUMM [0019] (2) analyzing the nucleotide sequence of the amplified hsp 65 gene fragment; and

SUMM [0020] (3) comparing the nucleotide sequence of the amplified hsp 65 gene fragment obtained in step (2) with a 604-bp hsp 65 gene fragment of a reference mycobacterial species.

SUMM [0021] It is a further object to provide a method for the detection or identification of mycobacterial species comprising the steps of:

SUMM [0022] (1) amplifying an hsp 65 gene fragment of mycobacteria of interest with primers for specifically amplifying the hsp 65 gene of mycobacteria ; and

SUMM [0023] (2) hybridizing the amplified hsp 65 gene fragment with a probe set comprising at least a probe of the hsp 65 gene fragment.

SUMM [0024] It is a further object to provide a method for the identification of mycobacterial species comprising the steps of amplifying an hsp 65 gene fragment of mycobacteria of interest with a pair of primers for specifically amplifying the hsp 65 gene of mycobacteria, and analyzing according to the Restriction Fragment Length Polymorphism (RFLP) analysis using the restriction enzyme recognition site in the amplified hsp 65 gene fragment.

SUMM [0025] It is a further object to provide a kit useful for the diagnosis or identification of mycobacterial species comprising a pair of primers for amplifying the hsp 65 gene of mycobacteria, and a restriction enzyme recognizing the restriction enzyme recognition site which is located in the amplified hsp 65 gene fragment.

SUMM [0026] It is a further object to provide a kit useful for the diagnosis or identification of mycobacterial species comprising an amplifying means comprising a pair of primers for specifically amplifying the 604-bp hsp 65 gene fragment of mycobacteria, a hybridization means comprising a probe set including at least a 604-bp hsp 65 gene fragment, and a labeling means for detecting the hybridized product.

DRWD [0028] FIG. 1 shows the hsp 65 gene fragment and the primers of the present invention;

DRWD [0029] FIG. 2 is a photograph of electrophoresis showing the amplified product of mycobacterial DNA, wherein panel A shows a result obtained from analysis of the amplified gene fragment of reference strains, and panel B shows a result for the amplified gene fragments of mycobacteria in a clinical sample;

DRWD [0030] FIG. 3 is a photograph of agarose gel electrophoresis showing the hsp 65 gene fragment of a reference mycobacterial species that was amplified and then treated with Xho I;

DRWD [0031] FIG. 4 is a summarized diagram showing a result of PCR-RFLP of hsp 65 gene fragments of reference strains of mycobacteria;

DRWD [0032] FIG. 5 is a photograph of agarose gel electrophoresis where hsp 65hsp 65 gene fragments of mycobacteria in a clinical sample were amplified and then treated with XhoI;

DRWD [0033] FIG. 6 shows phylogenetic relationships of 50 reference mycobacterial species obtained in Example 7; and

DRWD [0034] FIGS. 7a to 7d shows the results of the identification of mycobacteria in a clinical sample according to a comparative sequence analysis.

DETD [0035] The present invention relates to a pair of primers specific to mycobacteria, and more specifically to a pair of primers specifically amplifying an hsp 65 gene fragment of mycobacteria, an hsp 65 gene fragment, and a method for the identification of mycobacteria with the same.

DETD [0036] Considering the problems in conventional identification methods and the taxonomy of mycobacteria, the inventors provide PCR primers that can amplify M. tuberculosis and non-tuberculosis mycobacteria, an hsp 65 gene fragment as a chronometer molecule which exists in all mycobacteria, and a method for the identification of mycobacteria by using the primers and hsp 65 gene fragments. By using the restriction fragment of the amplified product of hsp 65 genes with treatment of Xho I, it is possible to differentiate M. tuberculosis and non-tuberculosis mycobacteria , and to differentiate non-tuberculosis mycobacteria.

DETD [0037] In order to obtain a pair of primers that preferably amplify the hsp 65 gene of mycobacteria, the inventors prepared the primers on the basis of the hsp 65 gene of M. tuberculosis (GenBank No. M15467), M. avium (GenBank No. AF281650) of which 1623-bp full sequences of the hsp 65 gene were analyzed, and T. paurometabola (GenBank No. AF352578) which is phylogenetically closer to mycobacteria. The forward primer comprises 20 nucleotides located at the 163rd position to the 182nd position of the hsp 65 gene sequence of the three mycobacteria , and the backward primer comprises 20 nucleotides located at the 787th position to the 806th position. In addition, the modified primers or polynucleotides comprising the primers can be used for amplifying 644-bp hsp 65 gene fragments of mycobacteria. The primer region of the hsp 65 gene is adopted from the region of M. tuberculosis and M. avium which belong to genus mycobacteria and Tsukamurella paurometabola. Preferably, the forward, primer is 5'-ATCGCCMGGAGATCGAGCT-3', which is called HSPF 3 and is shown in SEQ ID NO: 55. The backward primer is 5'-MGGTGCCGCGGATCTTGTT-3', which is called HSPR 4 and is shown in SEQ ID NO: 56. The positions of the hsp 65 gene fragment and the primers are schematically indicated in FIG. 1.

DETD [0038] The present invention provides polynucleotides of hsp 65 gene fragments used for detecting or identifying mycobacterial species. In addition, the present invention provides polynucleotide sets comprising at least a polynucleotide selected from the group consisting of hsp 65 gene fragments or complementary sequences thereto.

DETD [0039] The chronometer molecule used for the identification of mycobacterial species in the present invention is the 644-bp gene fragment located at the 163rd position to the 806th position of a 1623-bp hsp 65 gene of M. tuberculosis. The 644-bp gene fragment is substantially a 604-bp fragment because the 40-bp primer sequence is excluded. As a result of a Genbank database search, it was found that all 604-bp gene fragments of hsp 65 of 54 kinds of reference mycobacterial species are novel.

DETD [0040] To establish the database for detecting and identifying the mycobacteria, the reference strains as shown in Table 1 were employed. 50 reference strains included 47 reference strains from the American. . . the Catholic University of Korea, and 2 reference strains (type II, III) of M. kansasii from V. Vincent. In addition, hsp 65 gene fragments of 3 reference strains of

Tsukamurella from the German Collection of Microorganisms and Cell Cultures, and a reference. . . strain of Nocardia from ATCC were analyzed (Table 1).

TABLE 1

Reference strains of the present invention		
No	species	source
Reference strains of mycobacteria		
1	M. abscessus	CAP97E-03
2	M. africanum	ATCC 25420
3	M. asiaticum	ATCC 25276
4	M. aichiense	ATCC 27280
5	M. avium	ATCC 25291
6	M.. . . vaccae	ATCC 15483
48	M. wolinskyi	ATCC 700010
49	M. parafortuitum	ATCC 19686
50	M. farcinogenes	ATCC 35753
Reference strain of bacteria other than mycobacteria		
1	T. paurometabola	DSM 20162
2	T. tyrosinosolvens	DSM 44234
3	T. pulmonis	DSM 44142
4	N. carnea	ATCC 6847

DETD [0041] For detecting and identifying Mycobacterial species, the present invention provides 604-bp hsp 65 gene fragments as a new chronometer molecule, instead of 16S rDNA. The chronometer molecules must satisfy the following requirements in. . . between species. Thirdly, the target gene must have interspecies variation and intraspecies conservation, which suitably reflects a phylogenetic relationship. The hsp 65 gene fragment of the present invention suitably satisfies the requirements of the chronometer molecule.

DETD . . . M. tuberculosis (54 reference strains) have different nucleotide sequences, namely interspecies variation. In a previous report, the five kinds of mycobacteria belonging to the TB complex had the same nucleotide sequence analyzed according to another analyzing method using the 16S rDNA or rpoB gene fragment, and it was found that the mycobacterial species belonged to the same species. The result showed that the hsp 65 gene fragment of the present invention satisfied the interspecies variation of nucleotide sequences. Secondly, all 54 reference strains used in. . . the gap causes an error in establishing the phylogenetic tree at a high rate. Therefore, the identification method using the hsp 65 gene of the present invention provides significant advantages.

DETD [0043] In order to investigate whether the 604-bp hsp 65 gene fragment of the present invention can be suitable for use as a chronometer molecule, a phylogenetic tree was constructed by the nucleotide sequence of 604-bp hsp 65 gene fragments of various mycobacteria. In addition, the mycobacteria identified according to the other conventional method were analyzed by the identification method of the present invention using the hsp 65 gene fragment. As a result, the present invention accurately identified the mycobacteria.

DETD [0044] The phylogenetic tree of the reference strains of the present invention showed the natural relationships of the mycobacteria . That is, the result confirmed that 50 reference strains of TB complex formed a large group excluding T. paurometabola as an outgroup (FIG. 6).

Also, slow-growing mycobacteria and fast-growing mycobacteria formed different groups. M. tuberculosis and M. leprae of pathogenic mycobacteria formed the same branch of the phylogenetic tree. MOTT were isolated frequently. M. avium and M. intracellulare, showing quite similar biochemical characteristics, formed the same branch. The results showed general characteristics of mycobacteria. M. kansasii and M. gastri have 100% sequence homology, and thus cannot be differentiated according to the conventional identification method. . . but they are differentiated according to the present invention. Moreover, the subspecies of M. kansasii can be differentiated (namely, the hsp 65 gene fragments of M. kansasii Type I, II, and III have different nucleotide sequences). The results of the present invention show the phylogenetic relationships of mycobacteria. That is, the slow-growing mycobacteria and fast-growing bacteria form different branches of the phylogenetic tree, and M. tuberculosis and M. lepare form the same branch.

DETD [0045] The mycobacterial species can be identified according to the identifying method of mycobacteria, such as comparative sequence analysis, probe hybridization, and PCR-RFLP, using the polynucleotide of the present invention. The comparative sequence analysis,. . . the method which has been known to a skilled person in the art. For example, a method for identifying the mycobacteria with 16s rDNA can be applied for the identification method of the present invention.

DETD [0046] In one aspect, the present invention provides a method for the identification of mycobacteria by using PCR-RFLP (also called PRA). The method comprises the steps of amplifying hsp 65 gene fragments of mycobacteria with primers specific to mycobacteria, preferably primers as shown in SEQ ID NOs: 55 and 56, and analyzing the amplified product according to the RFLP analysis by. . . enzyme recognizing the target site located in the amplified product. The identification method is simple, economical, and specific to the mycobacteria.

DETD [0049] In comparison with the conventional method for identifying mycobacteria using the hsp 65 gene, the method of the present invention is simple and economical. The conventional method uses a 439-bp fragment of hsp 65 gene as a target gene, and two kinds of restriction enzymes, Hae II and BstE II. As described above, the. . . produced so that the small fragments, such as a 10-bp fragment, must be separated. Thus, in order to accurately identify mycobacteria in the conventional method, it is necessary to use the restriction fragment database of reference strains, or to analyze the mycobacteria of interest together with putative reference species according to restriction enzyme treatment and electrophoresis. In the preferred embodiment of the present invention, the identifying method of mycobacteria uses Xho-I recognizing six (6) nucleotides as target sites, thereby making it perform more gel electrophoresis. However, the identification method. . .

DETD [0050] The present invention provides a new system where a 644-bp hsp 65 gene fragment of mycobacteria is amplified with primers specifically for amplifying the 644-bp hsp 65 gene fragment of mycobacteria, and it is treated with Xho-I to differentiate and identify the mycobacterial species. Only a process of PRA makes it possible to differentiate the MOTT into 3 groups, as well as M.. . . That is, the treatment of the amplified product with a restriction enzyme produces only a 644-bp gene fragment in fast-growing mycobacteria, thereby differentiating it from the slow-growing mycobacteria. M. avium complex (for examples, M. avium and M. intracellulare) which belongs to slow-growing mycobacteria and is isolated most frequently in clinical samples produces three kinds of restriction fragments, 391 -bp, 169-bp, and 84-bp, thereby. . .

DETD [0051] Among the genus Mycobacterium that includes about 70 species, about 10 strains including M. tuberculosis, M. avium complex, M. kansasii, M. szulgai, M. gordonae,. . .

DETD [0052] In another aspect of the present invention, a TB complex can be differentiated from MOTT by treating the amplified hsp 65 gene fragment with Xho I, and analyzing it according to RFLP. In addition, the TB complex can be differentiated based on the restriction fragment of the amplified 644-bp hsp 65 fragments of 391 -bp, 150-bp, and 103-bp.

DETD [0053] In a further aspect of the present invention, a 644-bp hsp 65 gene fragment of fast-growing mycobacteria is not cleaved by a restriction enzyme, Xho I. The fast growing mycobacteria can be differentiated depending on whether the amplified product can be cleaved by the restriction enzyme or not. Thus, the present invention provides a method for differentiating fast-growing mycobacteria among MOTT. When 391 -bp, 169-bp, and 84-bp restriction fragments are produced by the treatment of Xho I and RFLP analysis of mycobacteria, the mycobacteria can be identified as species including M.avium, M. intracellulare, M.celatum, M. shimoidei, and M. szulgai.

DETD . . . In the case that the treatment of Xho I and the RFLP analysis produces 391-bp and 253-bp restriction fragments, the mycobacterial species are identified as species including M. gastri, M. genavense, M. gordonae, M. haemophilum, M. kansasii, M. malmoense, M. marinum,. . .

DETD [0056] The present invention also relates to a kit for differentiating or diagnosing mycobacterial species comprising Xho I and primers specific to the hsp 65 gene of mycobacterial species, preferably primers as shown in SEQ ID NOs: 55 and 56, wherein the DNA of mycobacterial species in a sample is amplified with the primers to produce the hsp 65 gene fragment, and the mycobacterial species are differentiated depending on the restriction fragments obtained according to RFLP. The kit further comprises a PCR amplification kit. . .

DETD [0057] In another aspect, the present invention relates to a method for detecting and identifying the mycobacterial species, comprising the steps of (1) amplifying 604-bp hsp 65 gene fragments of mycobacterial species of interest with a primer that can specifically amplify hsp 65 gene fragments, (2) hybridizing the amplified product with a probe set comprising at least a 604-bp hsp 65 gene fragment selected from the group consisting of the polynucleotide of a 604-bp hsp 65 gene fragment of mycobacterial species. In the embodiment of the method, the hsp 65 gene fragment of mycobacterial species of interest can be amplified according to general amplification methods of nucleotides such as PCR, LCR (ligase chain reaction),. . .

DETD [0058] The present invention provides an identification or diagnosis kit comprising (1) a means for amplification including a pair of primers specific to an hsp 65 gene of mycobacterial species; (2) a means for hybridization comprising a 604-bp or 644-bp gene fragment of hsp 65 of mycobacterial species, preferably a probe or probe set comprising a gene fragment selected from the group consisting of polynucleotides as shown. . .

DETD [0059] The present invention provides a method for identification of mycobacterial species by using comparative sequence analysis. The database of hsp 65 gene fragments as described above can be applicable to the method. The present invention provides a method for identification of mycobacterial species by using 604-bp hsp 65 gene fragments. More specifically, the method comprises the steps of:

DETD [0060] (1) amplifying hsp 65 gene fragments of

mycobacteria of interest with primers for specifically amplifying the hsp 65 gene of mycobacteria
;

DETD [0061] (2) analyzing the nucleotide sequence of the amplified hsp 65 gene fragment; and

DETD [0062] (3) comparing the nucleotide sequence of the amplified hsp 65 gene fragment obtained in step (2) with a 604-bp hsp 65 gene fragment of a reference strain of mycobacteria.

DETD [0063] Preferably, step (3) can be carried out by multi-aligning the 604-bp hsp 65 gene fragment of mycobacterial species of interest with a polynucleotide set comprising at least an hsp 65 604-bp polynucleotide of reference strains of mycobacteria to infer a phylogenetic tree. According to the comparative sequence analysis, a database of 604-bp hsp 65 gene fragments is established by amplifying the hsp 65 gene fragment of reference species of mycobacteria with the primers specific to mycobacteria, preferably primers as shown in SEQ ID NO: 55 and SEQ ID NO: 5, and analyzing the nucleotide sequence of the amplified. . . product. In the example of the present invention, the database of 604-bp fragments of 54 reference strains except for the primer sequence is established by analyzing the nucleotide sequences of the 604-bp fragments, and through multi-alignment. The 604-bp fragments of reference strains obtained in the present invention are shown in SEQ ID NO: 1 to SEQ ID NO: 54. The mycobacterial species of interest can be identified according to comparative sequence analysis by using the database.

DETD [0064] As the hsp 65 gene fragments of the mycobacterial species of interest are different from those of the reference species, mycobacterial species of interest can be identified based on the criterion of nucleotide sequence homology of hsp 65 genes of reference species. Because a mycobacterial species has a different range of sequence homology, mycobacterial species can be identified based on the specific range of the sequence homology thereof. For example, M. gordonae has a wide range of sequence homology, but M. tuberculosis has a narrow range. In addition, mycobacterial species can be identified by multi-aligning the nucleotide sequence of 604-bp hsp 65 gene fragments with those of reference species to infer a phylogenetic relationship.

DETD [0065] To confirm that the database including 604-bp hsp 65 gene fragments of 50 reference strains of mycobacterial species can be useful for identifying the mycobacterial species in a clinical sample, the identification method of the present invention was applied for 38 strains of mycobacteria obtained from the Korean Institute of Tuberculosis of the Korean National Tuberculosis Association, which had already been identified by using. . . at random. The biochemical method item is a result of identification by the Korean Institute of Tuberculosis, and the item hsp 65 gene analysis method is a result of the present invention.

TABLE 2

Identification result for clinical isolates

No.	analysis strain	Biochemical method	hsp 65 gene method
1	KIT 77009	M. tuberculosis	M. tuberculosis
2	KIT 77710	M. tuberculosis	M. tuberculosis

3 KIT M.. . .

DETD [0066] The nucleotide sequences of 38 mycobacteria obtained from the clinical sample were analyzed and then multi-aligned with the database of reference strains to infer the phylogenetic. . .

DETD . . . in public health. The results of the identification of M. tuberculosis by using the database of the reference species of mycobacteria of the present invention confirmed that all twenty (20) M. tuberculosis were identified (Table 2 and FIG. 7c), and showed that 604-bp hsp 65 gene fragments of 20 strains have 100% sequence homology with a 604-bp fragment of M. tuberculosis ATCC 27284 reference strain.. . . gene used as a target gene are involved in resistance to streptomycin and to rifampin, respectively. The target genes in mycobacteria with a resistance to antituberculosis drugs can be mutated. However, unlike 16s rDNA and rpoB, the hsp 65 gene is not related to resistance to antibiotics, and thus it does not mutate. Therefore, the 604-bp hsp 65 gene is stable with respect to the selection pressure of antituberculosis drugs in comparison with other target genes.

DETD . . . includes various genotypes, namely interspecies heterogeneity (Devallois A, Picardeau M, Paramasivan C N, Vincent V, Rastogi N: Molecular characterization of Mycobacterium avium complex isolates giving discordant results in AccuProbe tests by PCR-restriction enzyme analysis, 16s rRNA sequencing, and DT1-DT6 PCR. J. . .

DETD . . . reported to be separated from clinical material. 3 strains are identified as M. kansasii by using the database of 604-bp hsp 65 gene fragments of reference strains, which are consistent with results of the biochemical identification method. The method for identifying the mycobacterial species by using the database has characteristics such that M. kansasii can be differentiated from M. gastri, and subspecies of. . .

DETD [0076] As a result of identifying the clinically separated mycobacterial strains with the database of the present invention, 4 strains (KIT 32101, 32104, 32105, and 32106) were found to be M. gordonae (FIG. 7a, and Table 2). When comparing the nucleotide sequences of the 604-bp hsp 65 gene fragments of the 4 strains, they have 99.2-99.8% sequence homology with lo each other, but they have 95.9-96.3% sequence. . . the report that M. gordonae has intraspecies heterogeneity (Abed Y, Bollet C, de Micco P. Identification and strain differentiation of Mycobacterium species on the basis of DNA 16S-23S spacer region polymorphism. Res Microbiol. 1995 146(5): 405-13). That is, 4 isolates obtained. . .

DETD [0077] As a result of identification of mycobacterial species with the database of reference species, 4 strains (KIT 31102, 31103, 31106, and 31107) were identified as M. szulgai,. . .

DETD [0078] As a result of identification of mycobacterial species with the database of reference species, 1 strain was identified as M. marinum, which is consistent with that of. . .

DETD . . . in humans, and it includes 3 reference strains of the present invention (M. terrae, M. triviale, M. nonchromogenicum), and various mycobacterial species which are not classified. As a result of identification of mycobacterial species with the database of reference species, 4 strains were identified as M. nonchromogenicum among the M. terrae complex, which. . .

DETD [0080] F. Identification of Fast-growing Mycobacteria (M. fortuitum Complex and M. chelonae Complex)

DETD [0081] As a result of identification of mycobacterial species with the database of reference species, 2 strains (KIT 61104, 61105) were identified as M. abscessus of M. chelonae. . . problem of the conventional biochemical method that M. chelonae and M. abscessus cannot be differentiated. The nucleotide sequences of the hsp 65 gene fragments of the strains have 98.4-99.5% nucleotide sequence homology with M. abscessus CAP97E-03.

DETD . . . identified as M. fortuitum, which is consistent with the result of the biochemical identification method. M. fortuitum complex covers

various mycobacterial species, and includes M. fortuitum ATCC 6841, M. fortuitum ATCC 49403, M. fortuitum ATCC 49404, and M. peregrinum as reference. . .

DETD [0085] As shown in Table 1, The hsp 65 gene fragments of 50 reference strains were sequenced, including 47 reference strains from the American Type Culture Collection (ATCC), a. . .

DETD . . . strains and clinically isolated strains was extracted according to the Bead Beater Phenol (BB/P) extraction method. The culture of each mycobacteria was suspended with TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl: pH 8.0) placed in a 2.0. . . Tris-HCl, 1 mM EDTA), and used as a template DNA for the analysis of nucleotide sequence and identification of the mycobacterial species in the following Examples.

DETD Preparation of Primers for Amplifying hsp 65 Gene Fragments

DETD [0089] A forward primer and a backward primer were prepared for specifically amplifying hsp 65 genes of all mycobacterial species. hsp 65 genes of M. tuberculosis (GenBank No. M15467) and M. avium (GenBank No. AF281650) of which 623-bp full sequences were previously analyzed for another purpose, and T. paurometabola (GenBank No. AF352578) were used for this example to prepare primers for amplifying hsp 65 genes of all the mycobacteria. The primers were shown in SEQ ID NO: 55 and 56, and positions thereof are indicated in FIG. 1.

Forward primer: HSPF3
5'-ATCGCCAAGGAGATCGAGCT-3' (SEQ ID NO: 55)

Backward primer: HSPR4
5'-AAGGTGCCGCGGATCTTGTT-3' (SEQ ID NO: 56)

DETD Amplification of 644-bp hsp 65 Gene Fragment

DETD [0090] 3-1) PCR Amplification of hsp 65 Gene

DETD . . . (pH 8.3), and 1.5 mM MgCl.sub.2. 50 ng of each DNA isolated in Example 1, and 20 pmol of each primer prepared in Example 2 were placed in a tube and distilled water was added thereto to a final volume of. . .

DETD [0113] As shown in FIG. 2, 644-bp hsp 65 gene fragments were obtained from reference strains and clinically isolated strains used in the Example. Therefore, the result suggests that the primers of the present invention could amplify the hsp 65 gene of all the mycobacteria.

DETD Nucleotides Sequence Analysis of hsp 65 Gene Fragment

DETD [0115] Two strands of 604-bp hsp 65 gene fragments except for 40-bp of primer region which corresponded to the 183.sup.rd to 806.sup.th positions in hsp 65 of M. tuberculosis were sequenced with a forward primer (HSPF3) and a backward primer (HSPR4). The eluted DNA from the gel was used as a template, and automatic sequencing was performed. 1060 ng of the template DNA, 1.2 pmol of each primer, and 2 μl of dye from a BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems) were mixed, and distilled water. . . 3100 system (ABI3100, PE Applied Biosystems) after electrophoresis for 2 hours 30 min. From a search on Genbank, all 604-bp hsp 65 gene fragments of 54 reference strains were found to be novel.

DETD [0116] 4-2) Alignment of 604-bp hsp 65 Gene Fragment

DETD . . . obtained in the examples were multi-aligned by using the Megalign program of the Dnastar software to construct a database of hsp 65 gene fragments.

DETD . . . analyzed by EXAMPLE 4-2) were multi-aligned by using the Megalign program of the Dnastar software to construct a database of hsp 65 gene fragments.

DETD . . . amino acid residues were multiply aligned by a Clustal Method of the Megalign program. The database for identifying is the Mycobacteria was constructed using 604 bp nucleotides deduced from the aligned 301 amino acid residues. Sequence homology among nucleotide sequences of. . .

DETD . . . molecular evolutionary genetics analysis, version 1.01. Pennsylvania State University, University Park). The multiple aligned 604-bp polynucleotides from 50 kinds of mycobacterial species were used to construct a Neighbor-joining phylogenetic tree based on the Juke-Cantor distance estimation method and a pairwise deletion. . . 604-bp polynucleotide of T. paurometabola as a outgroup. An analysis of bootstrap was performed through 100 replications. 50 kinds of mycobacteria reference strains made a large group, and fast-growing mycobacteria and slow-growing mycobacteria were formed into different groups from each other. The result reflected the general characteristics of mycobacteria in that pathogenic mycobacteria, M. tuberculosis, and M. leprae were located in the same branch, and M. avium and M. intracellulare among MOTT were. . .

DETD Differentiation of Reference Strains of Mycobacteria by Using the PRA

DETD . . . (5'-CTCGAG-3') with 6 nucleotide recognition sites was determined by analyzing 644-bp (corresponding to the 163r to the 806.sup.th position) of hsp 65 gene of M. tuberculosis (GenBank No. M15467) and M. avium (GenBank No. AF281650) with the Mapdraw program of Dnastar software.

DETD . . . Source

TB complex		
1	M. africanum	ATCC 25420
2	M. bovis	ATCC 19210
3	M. bovis BCG	French strain
4	M. tuberculosis H37Rv	ATCC 27294
Slow-growing mycobacteria		
5	M. avium	ATCC 25291
6	M. celatum Type I	ATCC 51131
7	M. celatum Type II	ATCC 51130
8	M. gastri	ATCC 15754
9. . .	M. scrofulaceum	ATCC 19981
18	M. shimoidei	ATCC 27962
19	M. simiae	ATCC 25275
20	M. szulgai	ATCC 35799
21	M. ulcerans	ATCC 19423
Rapid-growing mycobacteria		
22	M. abscessus	CAP97E-03
23	M. chelonae	ATCC 35749
24	M. chitae	ATCC 19627
25	M. fortuitum 49403	ATCC 49403
26	M. fortuitum 6841	ATCC. . .

DETD . . . reference strains (M. tuberculosis, M. bovis, M. bovis BCG, M. africanum) which belong to a TB complex of strongly pathogenetic mycobacteria could be differentiated from opportunistic pathogens of MOTT by use of specific restriction fragments of 391-bp, 150-bp, and 103-bp. The. . .

DETD [0127] FIG. 3 is a photograph of agarose gel electrophoresis of a 644-bp PCR product of an hsp 65 gene fragment of reference strain treated with Xho I.

DETD [0138] Differentiation of Fast-growing Mycobacteria

DETD [0139] Lanes 12-16 in FIG. 3 indicated that 644-bp fast-growing mycobacteria including M. fortuitum 6841, M. abscessus, M. chelonae, and M. peregrinum were not cleaved by Xho-I, thereby differentiating them from the other mycobacteria (FIGS. 3 and 4).

DETD Differentiation of Clinically Isolated Strains of Mycobacteria

by Using the PRA
DETD . . . Table 4.
TABLE 4

PRA analysis of clinically isolated strains

strain	No. of isolates
TB complex	
M. tuberculosis	54
M. bovis	9
Slow-growing mycobacteria	
M. avium complex	49
M. kansasii	30
M. szulgai	12
M. gordonae	9
M. marinum	3
Rapid-growing mycobacteria	
M. fortuitum	17
M. chelonae	15
Sum	198

DETD [0143] FIG. 5 is a photograph of 2% agarose gel electrophoresis of a reaction product obtained by treating the amplified hsp 65 gene fragment with Xho I. In panel A, lane M is a DNA size marker obtained by treating 174 with. . .

DETD . . . restriction fragments of 391 bp, 150bp, and 103-bp, thereby differentiating them from 144 strains of MOTT. 32 strains of fast-growing mycobacteria were not cleaved by the restriction enzyme, so they could be differentiated from the other 168 strains. 49 clinical isolates. . .

DETD [0145] This example confirmed that the PRA method of the present invention by using the hsp 65 gene can be applied to a clinical isolate of mycobacteria.

DETD [0146] As shown in Table 2, 38 mycobacterial species including 10 kinds of TB complex and 28 MOTT obtained from the Koran Institute of Tuberculosis (Seoul, Korea) were. . .

DETD [0147] DNA extraction, amplification, and PCR-mediated sequencing of hsp 65 gene fragments were accomplished according to the methods described in Examples 3 and 4. Then, the result was multi-aligned with. . .

DETD . . . (KIT 41110) had 99.5% nucleotide sequence homology with M. avium ATCC 25281 which included 3 different nucleotides in a 604-bp hsp 65 gene fragment. When the nucleotide sequences of 3 strains of M. intracellulare (KIT 41105, 41111, and 51115) were compared with. . .

DETD . . . and 32106) were identified as M. gordonae (FIG. 7a and Table 2). When comparing the nucleotide sequences of a 604-bp hsp 65 gene fragment of 4 clinically isolated strains, they had 99.2-99.8% sequence homology with each other, but they had 95.9-96.3% sequence. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 1
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium abscessus
SEQUENCE: 1
ggaggacccg tacgagaaga tcggcgctga gctggtcaag gaagttgcca agaagaccga 60
cgacgtcgcg ggtgacggca ccaccaccgc caccgtgctc gcccaggctc tggtcaagga 120
aggtctgcgt aacgtcgccg ccggcgccaa cccgctcggc. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium africanum

SEQUENCE: 2
ggaggatccg tacgagaaga tcggcgccga gctggtcaaa gaggtagcca agaagaccga 60
tgacgtcgcc ggtgacggca ccacgacggc caccgtgctg gcccaggcgt tggttcgcga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium asiaticum
SEQUENCE: 3
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtggcc ggtgacggca ccacgacggc caccgtgctg gcacaggcgc tggtcaagga 120
gggcctgcgc aacgttgccg caggcgccaa cccgctgggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium aichiense
SEQUENCE: 4
cgaggacccg tacgagaaga tcggcgctga gctggtcaag gaagtcgcca agaagactga 60
cgatgtcgcg ggcgacggca ccaccaccgc caccgtgctc gctcaggctc tggttcgcga 120
aggtctgcgc aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium avium
SEQUENCE: 5
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca cgacgacggc cacggtgctc gcccaggcgt tggtccgcga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctgggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium bovis
SEQUENCE: 6
ggaggatccg tacgagaaga tcggcgccga gctggtcaaa gaggtagcca agaagaccga 60
tgacgtcgcc ggtgacggca ccacgacggc caccgtgctg gcccaggcgt tggttcgcga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterim celatum Type 1
SEQUENCE: 8
ggaggacccc tacgaaaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcg ggtgacggta cgacgacggc cacggtgctg gcccaggcgc tggtcaagga 120
gggcctgcgc aacgtcgccg. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 10
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium chelonae
SEQUENCE: 10
ggaggacccg tacgagaaga tcggcgctga gctggtcaag gaagttgcca agaagactga 60
cgacgtcgcg ggtgacggca ctactaccgc caccgtgctt gcccaggctc tggtcaagga 120
aggtctgcgt aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 11
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium chitae
SEQUENCE: 11

ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagactga 60
cgacgtcgcc ggcgacggca ccaccaccgc caccgttctg gcccaggcgc tggttcgcga 120
aggtctgcgc aacgtcgcgg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 12
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium microti
SEQUENCE: 12
ggaggatccg tacgagaaga tcggcgccga gctggtcaaa gaggtagcca agaagaccga 60
tgacgtcgcc ggtgacggca ccacgacggc caccgtgctg gcccaggcgt tggttcgcga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 15
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium fortuitum 49403
SEQUENCE: 15
ggaggacccg tacgagaaga tcggcgctga gctcgtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcg ggcgacggca ccaccaccgc caccgttctg gcccaggccc tggttcgcga 120
aggtctgcgc aacgtcgctg ccggcgccaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 16
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium fortuitum 49404
SEQUENCE: 16
ggaggacccg tacgagaaga tcggcgcaga gctggtcaag gaagtcgcca agaagactga 60
cgacgtcgca ggcgacggca ccaccacggc caccgtgctc gcccaggctc tggttcgcga 120
aggtctgcgc aacgtcgcag ccggcgccaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 17
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium gastri
SEQUENCE: 17
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggcgacggca ccaccacggc caccgtgctc gcgcaggcgc tggtcaagga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctgggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 18
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium genavense
SEQUENCE: 18
ggaggacccc tacgagaaga tcggcgctga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca ccacgacggc caccgtgctc gctcaggcgc tcgtcaagga 120
gggcctgcgc aacgtggcgg ccggcgccaa cccgctgggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 603
TYPE: DNA
ORGANISM: Mycobacterium gordonae
SEQUENCE: 19
gaggacccgt acgagaagat cggcgctgag ctggtcaagg aagtcgccaa gaagaccgac 60
gacgttgccg gcgacggcac gacgacggcg accgtgctgg cgcaggcact ggtcaaggaa 120
ggcctgcgca acgtagccgc cggcgccaac ccgctggggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium haemophilum
SEQUENCE: 20
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60

cgacgtcgct ggtgatggca ccacgacggc gacggtgctg gctcaggcgc tggtcaaaga 120
gggcctgcgt aacgtcgcgg ccggcgccaa cccgctgggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 21
LENGTH: 603
TYPE: DNA
ORGANISM: Mycobacterium interjectum
SEQUENCE: 21
gaggacccgt acgagaagat cggcgccgag ctggtcaagg aagtcgccaa gaagaccgac 60
gacgtcgccg gtgacggcac gacgacggcc acggtgctgg cccaggccct ggtcaaggag 120
ggcctgcgca acgtcgcggc cggcgccaac ccgccggcgc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium intermedium
SEQUENCE: 22
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagttgcca agaagacgga 60
cgacgtcgcc ggtgacggca ccacgacggc caccgtgctc gcccaggcgc tggtgcgcga 120
gggtctgcgc aatgtcgctg ccggtgccaa cccgctgagc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 23
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium intracellulare
SEQUENCE: 23
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca cgacgacggc cacggtgctg gctcaggcgt tggtccgcga 120
gggcctgcgt aacgtcgccg ccggcgccaa cccgctgggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium leprae
SEQUENCE: 27
ggaggacccg tacgagaaga ttggcgctga gttggtcaag gaagtcgcca agaagacaga 60
tgacgtcgcc ggtgatggca ccacgacggc caccgtgctg gcccaggcat tggtcaaaga 120
gggcctacgc aacgtcgcgg ccggcgccaa cccgctaggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium malmoense
SEQUENCE: 28
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtggcc ggtgacggca cgacgacggc caccgtgctg gcgcaggcgc tggtcaaaga 120
gggcctgcgc aacgtcgcgg ccggtgccaa cccgctcagc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium marinum
SEQUENCE: 29
ggaggacccg tacgagaaga tcggcgctga gctggtcaag gaagttgcca agaagaccga 60
cgacgtggcc ggtgacggca cgacgacggc caccgtgctg gcccaggcgc tggtcaagga 120
aggcctgcgc aacgttgcgg ccggtgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium mucogenicum
SEQUENCE: 30
ggaggacccg tacgagaaga tcggcgctga gctggtcaag gaagttgcca agaagacgga 60
cgacgtcgct ggcgacggca ccaccaccgc caccgtgctg gcccaggccc tggttcgcga 120

aggcctgcgc aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium neoaurum
SEQUENCE: 31
ggaggacccg tacgagaaga tcggcgccga gctggtcaaa gaggtcgcca agaagaccga 60
tgacgtcgcg ggcgacggca ccaccaccgc caccgtgctg gcccaggccc tggttcgcga 120
aggtctgcgc aacgtcgcgg ccggcgccaa ccccctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium nonchromogenicum
SEQUENCE: 32
ggaggatccc tacgagaaga tcggcgctga gctggtcaaa gaggtcgcca agaagactga 60
cgacgtcgcg ggtgacggca ccaccaccgc caccgtgctc gcccaggccc tggtcaagga 120
aggcctgcgc aacgtggccg ccggcgccaa cccgctgggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 35
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium peregrinum
SEQUENCE: 35
ggaggacccg tacgagaaga tcggcgctga gctggtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcg ggtgacggca ccaccaccgc caccgttctg gcccaggccc tggttcgcga 120
aggtctgcgc aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 36
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium scrofulaceum
SEQUENCE: 36
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca cgacgacggc cacggtgctg gcccaggcgc tggtcaagga 120
gggcctgcgc aacgtcgcgg cgggcgccaa cccgctgagc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 37
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium senegalense
SEQUENCE: 37
ggaggacccg tacgagaaga tcggcgctga gctggtcaag gaagtcgcca agaagactga 60
cgacgtcgcg ggtgacggca ccaccaccgc caccgttctg gcccaggccc tggttcgtga 120
aggtctgcgt aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 38
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium shimoidei
SEQUENCE: 38
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca ccaccaccgc caccgtgctg gcccaggcgc tggtccacga 120
ggggctgcgc aacgtcgcgg ccggtgccaa cccgctcagc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 39
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium simiae
SEQUENCE: 39
ggaggacccc tacgagaaga tcggcgctga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggtgacggca ccacgacggc caccgtgctc gctcaggcgc tcgtcaagga 120
gggcctgcgc aacgtggcgg ccggcgccaa cccgctgggc. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium smegmatis
SEQUENCE: 40
cgaggacccc tacgagaaga tcggtgctga gctcgtcaaa gaggtcgcca agaagaccga 60
cgatgtcgct ggcgacggca ccaccaccgc caccgtcctg gctcaggccc tggttcgcga 120
aggcctgcgc aacgtcgctg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium szulgai
SEQUENCE: 41
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagttgcca agaagaccga 60
cgacgtcgcc ggtgacggca cgacgacggc caccgtgttg gcccaggcgc tggtcaagga 120
gggcctgcgc aacgtagcgg ccggcgccaa cccgctgggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium terrae
SEQUENCE: 42
ggaggacccc tacgagaaga tcggcgccga gctggtcaaa gaggtcgcca agaagaccga 60
cgatgtcgcc ggtgacggca ccaccacggc caccgtgctg gcacaggcgc tggtcaagga 120
aggcctgcgc aacgtggccg ccggcgccaa cccgctggcc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 43
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium thermoresistibile
SEQUENCE: 43
ggaggacccc tacgagaaga tcggcgctga gctggtcaag gaagtcgcca agaagaccga 60
cgacgtcgcc ggcgacggca ccaccaccgc caccgtcctg gctcaggcgc tggtgaagga 120
aggtttgcgc aacgtcgcgg ccggggccaa cccgctcgct. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium triviale
SEQUENCE: 44
ggaggacccg tacgagaaga tcggcgccga gctggtcaag gaagtcgcca agaagaccga 60
cgatgtcgcc ggtgacggca ccaccacggc caccgtgctc gcccaggcgc tggtgcgcga 120
gggcctgcgc aacgtcgccg cgggcgccaa cccgatgggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 45
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium tuberculosis
SEQUENCE: 45
ggaggatccg tacgagaaga tcggcgccga gctggtcaaa gaggtagcca agaagaccga 60
tgacgtcgcc ggtgacggca ccacgacggc caccgtgctg gcccaggcgt tggttcgcga 120
gggcctgcgc aacgtcgcgg ccggcgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium ulcerans
SEQUENCE: 46
ggaggacccg tacgagaaga ttggcgctga gctggtcaag gaagttgcca agaagaccga 60
cgacgtggcc ggtgacggca cgacgacggc caccgtgctg gcccaggcgc tggtcaagga 120
aggcctgcgc aacgttgcgg ccggtgccaa cccgctcggt. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 47
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium vaccae
SEQUENCE: 47
ggaggacccg tacgagaaga tcggcgctga gctggtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcg ggcgacggta ccaccaccgc caccgtgctc gctcaggctc tggttcgcga 120
aggcctgcgc aacgtcgcag ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 48
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium wolinskyi
SEQUENCE: 48
ggaggacccg tacgagaaga tcggcgctga gctggtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcc ggcgacggca ccaccaccgc caccgttttg gcccaggctc tggttcgcga 120
aggtctgcgc aacgtcgcgg ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 49
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium parafortuitum
SEQUENCE: 49
ggaggacccg tacgagaaga tcggcgctga gctggtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcg ggcgacggca ccaccaccgc caccgtgctc gctcaggccc tggttcgcga 120
aggtctgcgc aacgtcgcag ccggcgccaa cccgctcggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 50
LENGTH: 604
TYPE: DNA
ORGANISM: Mycobacterium farcinogenes
SEQUENCE: 50
ggaggacccg tacgagaaga tcggcgctga gctcgtcaaa gaggtcgcca agaagaccga 60
cgacgtcgcg ggcgacggca ccaccaccgc caccgttctg gcccaggccc tggttcgcga 120
aggtctgcgc aacgtcgctg ccggcgccaa cccgctcggc. . .
CLM What is claimed is:

1. A pair of primers for specifically amplifying an hsp 65 (Heat Shock Protein 65) gene fragment of mycobacterial species comprising the nucleotide sequences as shown in SEQ ID NO: 55 and SEQ ID NO: 56.

2. A polynucleotide of an hsp 65 gene fragment of mycobacterial species wherein the fragment is amplified by using a pair of primers specifically amplifying the hsp 65 gene fragment of mycobacterial species comprising the nucleotide sequences as shown in SEQ ID NO: 55 and SEQ ID NO: 56.

4. A polynucleotide set for the detection or identification of mycobacterial species wherein the set comprises at least an hsp 65 gene fragment selected from the group consisting of the polynucleotides as shown in SEQ ID NO: 1 to SEQ ID. .

5. A method for the identification of mycobacterial species comprising the steps of: (1) amplifying an hsp 65 gene fragment of mycobacterial species of interest with primers for specifically amplifying the hsp 65 gene fragment; (2) analyzing a nucleotide sequence of the amplified hsp 65 gene fragment; and (3) comparing the nucleotide sequence of the amplified hsp 65 gene fragment obtained in step (2) with a 604-bp hsp 65 gene fragment of a reference mycobacterial species.

6. The method of claim 5, wherein the primers comprise the polynucleotides as shown in SEQ ID NO: 55 and SEQ ID NO: 56.

7. The method of claim 5, wherein step (3) of comparing the nucleotide sequence of the mycobacterial species of interest with that of a reference mycobacterial species is performed by multi-aligning the nucleotide sequence of the 604-bp hsp 65 gene fragment of the mycobacterial species of interest with a polynucleotide set of claim 4 to infer a phylogenetic tree.

8. A method for the identification of mycobacterial species comprising the steps of: (1) amplifying an hsp 65 gene fragment of mycobacterial species with primers of claim 1; and (2) analyzing the amplified fragment according to the RFLP (Restriction Fragment Length Polymorphism) analysis method using. . .

10. The method of claim 9 comprising the step of treating the amplified hsp 65 gene fragment with Xho I to produce restriction fragment(s), and analyzing the restriction fragment(s) according to an RFLP analysis method to differentiate TB complex (Mycobacterium tuberculosis complex) and MOTT (Mycobacteria other than Mycobacterium tuberculosis).

12. The method of claim 10, wherein the 644-bp hsp 65 gene fragment is not cleaved by a restriction enzyme to identify fast-growing mycobacteria of MOTT.

13. The method of claim 10, wherein the restriction fragments are 391-bp, 169-bp, and 48-bp to identify a mycobacterial species selected from the group consisting of M, avium, M. intracellulare, M. celatum, M. shimoidei, and M. szulgai.

14. The method of claim 10, wherein the restriction fragments are 391-bp and 253-bp to identify a mycobacterial species selected from the group consisting of M, gastri, M. genavense, M. gordonae, M. haemophilum, M. kansasii, M. malmoense, M.. . .

15. A kit for the differentiation or diagnosis of TB complex and MOTT comprising a pair of primers of claim 1 and Xho I, wherein the mycobacterial species is differentiated or diagnosed based on the size of restriction fragment(s) which is obtained by amplifying an hsp 65 gene fragment of mycobacterial species in a sample with the primers to produce an amplified fragment and analyzing the amplified fragment according to an RFLP analysis method.

16. A method for the identification of a mycobacterial species comprising the steps of: (1) amplifying an hsp 65 gene fragment of a mycobacterial species of interest with primers for specifically amplifying an hsp65 gene of mycobacteria; and (2) hybridizing the amplified hsp65 gene fragment with a probe set comprising at least a probe selected from the. . .

L12 ANSWER 4 OF 5 USPATFULL on STN
AN 1999:150924 USPATFULL
TI Universal targets for species identification
IN Goh, Swee Han, Vancouver, Canada
Chow, Anthony, W. Vancouver, Canada
Hemmingsen, Sean, Saskatoon, Canada
PA University of British Columbia, Vancouver, Canada (non-U.S. corporation)
The National Research Council of Canada, Ottawa, Canada (non-U.S. corporation)
PI US 5989821 19991123
AI US 1997-3067 19970105 (9)
RLI Division of Ser. No. US 1995-429121, filed on 26 Apr 1995, now patented, Pat. No. US 5708160, issued on 13 Jan 1998

DT Utility
FS Granted
EXNAM Primary Examiner: Fredman, Jeffrey
LREP Fish & Richardson P.C.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1216
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides oligonucleotide primers and a method of using these primers for identification of the species of an organism, wherein the identification includes amplification of a variable polynucleotide sequence encoding a highly conserved region of a heat shock polypeptide.

AB The present invention provides oligonucleotide primers and a method of using these primers for identification of the species of an organism, wherein the identification includes amplification of a variable polynucleotide sequence encoding a. . .

SUMM This invention relates generally to taxonomic and phylogenic identification of organisms and specifically to the use of universal oligonucleotide primers and HSP60 amplicons to identify and distinguish organisms at the species level.

SUMM Three studies have reported the use of various Mycobacterium genus specific primers to amplify various target regions of the HSP 65 genes of Mycobacteria via PCR (Hance, et al., Mol. Microbiol., No. 3, 7:843-849, 1989; Plikaytis, et al., J. Clin. Microbiol., 30:1815-1822, 1992; Telenti,. . .

SUMM Two other studies (Plikaytis, et al., supra; Telenti, et al., supra) described similar PCR strategies for Mycobacterial speciation, but these methods required the detection of restriction enzyme site polymorphisms (RFLP) within the PCR amplified products. Both methods relied on the use of two restriction enzymes for differentiating the Mycobacterial species. Also, intraspecies DNA RFLPs were observed. En initial studies with clinical samples (Telenti, et al., supra), problems were observed. . .

SUMM . . . and classification of organisms. The identification of highly conserved regions flanking a variable region led to the production of universal primers which can be used to specifically amplify these variable regions of nucleic acid, thereby providing a target sequence for use. . .

SUMM The primers and the method of the invention are useful for the identification of organisms, including pathogens and non-pathogens, isolated from human/animal,. . .

DRWD . . . as H279) and 3' (designated as H280) flanking region of HSP60 for over 40 different organisms, to which the oligonucleotide primers of the invention hybridize.

DETD The present invention provides oligonucleotide primer(s) for identification of a organism wherein the identification includes amplification of variable regions of a polynucleotide sequence encoding a heat. . .

DETD In a first embodiment, the present invention provides isolated oligonucleotide primer(s) for identification of an organism wherein the identification includes amplification of a polynucleotide sequence encoding a region of a heat. . .

DETD . . . B. subtilis, Streptococcus faecalis, Bartonella henselae, B. quintana, B. bacilliformis, Yersinia pseudotuberculosis, Vibrio cholera, Legionella pneumophila, Helicobacter pylori, Neisseria gonorrhoeae, Mycobacterium marinum, Candida albicans, and P. aeruginosa as well as organisms listed in FIGS. 1 and 4 herein.

DETD The identification of a species of organism is accomplished by oligonucleotide(s) which are primers for amplification of the highly conserved region of a genomic locus having the sequence of a heat shock protein. These unique oligonucleotide primers were produced based upon identification of the flanking regions contiguous

with a region of the heat shock protein, HSP60, locus. These oligonucleotide primers comprise sequences which are capable of hybridizing with the flanking nucleotide sequence having substantially the sequence:

DETD . . . under stringent conditions and sequences having sufficient homology with SEQ ID NO:1 and SEQ ID NO:2, such that the oligonucleotide primers of the invention hybridize to the sequence.

DETD The primers of the invention embrace oligonucleotides of sufficient length and appropriate sequence so as to provide specific initiation of polymerization on a significant number of nucleic acids in the polymorphic locus. Specifically, the term "primer" as used herein refers to a sequence comprising two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and most. . . presence of nucleoside triphosphates and an agent for polymerization, such as DNA polymerase, and a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The oligonucleotide primer typically contains 12-20 or more nucleotides, although it may contain fewer nucleotides.

DETD Primers of the invention are designed to be "substantially" complementary to each strand of the genomic locus to be amplified. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions which allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with the 5' and 3' flanking sequences to hybridize therewith and permit amplification of the genomic. . . 5' and 3' flanking sequences for the genomic locus of over 40 different prokaryotic species which are amplified by the primers of the invention. Preferably, the primers of the invention include:

DETD where A is adenosine, T is thymidine, C is cytosine, G is guanosine and I is inosine. Primers having substantial homology to SEQ ID NO:3 and SEQ ID NO:4 are also included in the present invention.

DETD Oligonucleotide primers of the invention are employed in the amplification process which is an enzymatic chain reaction that produces exponential quantities of polymorphic locus relative to the number of reaction steps involved. Typically, one primer is complementary to the negative (-) strand of the polymorphic locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA Polymerase I (Klenow). . . and -strands containing the target polymorphic locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension results in exponential production of the region (i.e., the target polymorphic locus sequence) defined by the primer. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

DETD The oligonucleotide primers of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments. . .

DETD . . . may also be employed, or the nucleic acids produced in a previous amplification reaction herein, using the same or different primers may be so utilized. The specific nucleic acid sequence to be amplified, i.e., the polymorphic locus, may be a fraction. . .

DETD . . . as the template. Strand separation can be effected either as a separate step or simultaneously with the synthesis of the primer

extension products. This strand separation can be accomplished using various suitable denaturing conditions, including physical, chemical, or enzymatic means, the. . .

DETD . . . acid containing the sequence to be amplified is single stranded, its complement is synthesized by adding one or two oligonucleotide primers. If a single primer is utilized, a primer extension product is synthesized in the presence of primer, an agent for polymerization, and the four nucleoside triphosphates described below. The product will be partially complementary to the single-stranded. . . unequal length strands that may then be separated into single strands to produce two single separated complementary strands. Alternatively, two primers may be added to the single-stranded nucleic acid and the reaction carried out as described.

DETD . . . as a template for the synthesis of additional nucleic acid strands. This synthesis is performed under conditions allowing hybridization of primers to templates to occur. Generally synthesis occurs in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. Preferably, a molar excess (for genomic nucleic acid, usually about 10.sup.8 :1 primer:template) of the two oligonucleotide primers is added to the buffer containing the separated template strands. It is understood, however, that the amount of complementary strand. . . may not be known if the process of the invention is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter, however, the amount of primer added will generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified. . .

DETD The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90°-100° C. from about 1 to 10 minutes, preferably. . . 4 minutes. After this heating period, the solution is allowed to cool to room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art.. . .

DETD The agent for polymerization may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, E. coli DNA polymerase I, Klenow fragment of. . . polymerase, other available DNA polymerases, polymerase muteins, reverse transcriptase, and other enzymes, including heat-stable enzymes (i.e., those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation). Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each polymorphic locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may. . .

DETD The above process is repeated on the single-stranded molecules. Additional agent for polymerization, nucleotides, and primers may be added, if necessary, for the reaction to proceed under the conditions prescribed above. Again, the synthesis will be initiated at one end of each of the oligonucleotide primers and will proceed along the single strands of the template to produce additional nucleic acid. After this step, half of the extension product will consist of the specific nucleic acid sequence bounded by the two primers.

DETD Now that the present invention has provided novel oligonucleotide

primers for the amplification of a variable genomic region, the invention provides a method for the identification of the species of an organism comprising amplifying a region of the genomic nucleic acid of the organism by means of oligonucleotide primers which hybridize to target flanking 5' and 3' polynucleotide sequences of the genomic nucleic acid, the target polynucleotide sequence having. . .

DETD . . . amplification have been described and can also be employed as long as the HSP60 locus amplified by PCR using the primers of the invention is similarly amplified by the alternative means. Such alternative amplification systems include but are not limited to. . . acid amplification technique is nucleic acid sequence-based amplification (NASBA) which uses reverse transcription and T7 RNA polymerase and incorporates two primers to target its cycling scheme. NASBA can begin with either DNA or RNA and finish with either, and amplifies to. . . Alternatively, nucleic acid can be amplified by ligation activated transcription (LAT). LAT works from a single-stranded template with a single primer that is partially single-stranded and partially double-stranded. Amplification is initiated by ligating a cDNA to the promoter oligonucleotide and within. . . fills and joins the gap, mimicking normal DNA repair. Nucleic acid amplification by strand displacement activation (SDA) utilizes a short primer containing a recognition site for HincII with a short overhang on the 5' end which binds to target DNA. A DNA polymerase fills in the part of the primer opposite the overhang with sulfur-containing adenine analogs. HincII is added but only cut the unmodified DNA strand. A DNA polymerase that lacks 5' exonuclease activity enters at the site of the nick and begins to polymerize, displacing the initial primer strand downstream and building a new one which serves as more primer. SDA produces greater than 10.sup.7 -fold amplification in 2 hours at 37° C. Unlike PCR and LCR, SDA does not. . .

DETD Another embodiment of the invention provides a target genomic polynucleotide locus which is defined by being amplified by the primers of the invention identified by SEQ ID NO:3 and SEQ ID NO:4, or primer sequences substantially complementary thereto, wherein the polynucleotide locus does not hybridize with a polynucleotide locus from Staphylococcus aureus amplified by primers of the invention identified by SEQ ID NO:3 and SEQ ID NO:4, or primer sequences substantially complementary thereto. The genomic locus defined by amplification of the primers as described herein encodes a heat shock polypeptide, e.g., HSP60.

DETD . . . one of the container means may comprise means for amplifying target DNA, said means comprising the necessary enzyme(s) and oligonucleotide primers for amplifying said target DNA from the organism or a cell of the organism. The oligonucleotide primers include primers having a sequence:

DETD or primer sequences substantially complementary thereto. The target flanking 5' and 3' polynucleotide sequence has substantially the sequence selected from the group. . .

DETD . . . 50 ng of genomic DNA, 2 U of Taq DNA polymerase (GIBCO) and 0.5 μg of each of the degenerate primers H279 (SEQ ID NO:3) and H280 (SEQ ID NO:4). A final volume made up to 100 μl with dH.sub.2 O. . .

DETD The sequences of the two primers were 5'-GAIIIIGCIGGIGA(TC)GGIACIACIAC-3' (SEQ ID NO:3) and 5'-(T/C)(T/G)I(T/C)(T/G)ITCICC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ID NO:4), for H279 and H280, respectively. Inosine (I) was used to reduce the degeneracy of the primers. The last 26 nucleic acid residues of primers H279 and H280 correspond to DNA residue numbers 688 to 713 and the complement of residue numbers 1267 to 1292,. . .

DETD . . . protocols. Labelling of the 600 bp fragments for use as probes was carried out using Digoxigenin-11-dUTP and the standard random primer method (Maniatis, et al., supra, Molecular Cloning: A Laboratory Manual; Boehringer Mannheim protocols).

DETD FIG. 2 shows the results of experiments used to determine (i) if the degenerate primers could be used to amplify specific Staphylococcal targets from mixed cultures and (ii) if species specific HSP 60 probes identify. . .

DETD The degenerate primers of the invention were capable of amplifying a 600 bp putative HSP 60 fragment from all Staphylococci species and subspecies listed in FIG. 4. Thus, species specific probes can be easily generated with such primers. The Examples presented herein concentrated specifically on six species responsible for the majority of clinical Staphylococcal infections (Kloos and Lamba,. . .

DETD . . . targeted organisms in clinical samples, especially those from normally sterile sites such as cerebrospinal fluid and sera. The degenerate PCR primers have been used successfully as illustrated herein, to amplify the expected 600 bp HSP 60 fragment from diverse organisms. Though. . .

CLM What is claimed:

. . . of an organism comprising: a) amplifying a region of a genomic nucleic acid of the organism by means of oligonucleotide primers which hybridize to target flanking 5' and 3' polynucleotide sequences of the genomic nucleic acid, the target polynucleotide sequence having. .

3. The method of claim 1, wherein the primer is 5'-GAIIIIGCIGGIGA(T/C)GGIACIACIAC-3' (SEQ ID NO:3) or 5'-(T/C)(T/G)I(T/C)(T/G)ITCICC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ED NO:4).

L12 ANSWER 5 OF 5 USPATFULL on STN

AN 1998:4758 USPATFULL

TI HSP-60 genomic locus and primers for species identification

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PI US 5708160 19980113

AI US 1995-429121 19950426 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Fredman, Jeffrey

LREP Fish & Richardson, P.C.

CLMN Number of Claims: 10

ECL Exemplary Claim: 1,3

DRWN 9 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 1165

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides oligonucleotide primers and a method of using these primers for identification of the species of an organism, wherein the identification includes amplification of a variable polynucleotide sequence encoding a highly conserved region of a heat shock polypeptide.

TI HSP-60 genomic locus and primers for species identification

AB The present invention provides oligonucleotide primers and a method of using these primers for identification of the species of an organism, wherein the identification includes amplification of a variable polynucleotide sequence encoding a. . .

SUMM This invention relates generally to taxonomic and phylogenic identification of organisms and specifically to the use of universal oligonucleotide primers and HSP60 amplicons to identify and distinguish organisms at the species level.

SUMM Three studies have reported the use of various Mycobacterium genus specific primers to amplify various target regions of the HSP 65 genes of Mycobacteria via PCR (Hance, et al., Mol. Microbiol., No.3, 7:843-849, 1989; Plikaytis, et

al., J. Clin. Microbiol., 30:1815-1822, 1992; Telenti, et. . .

SUMM Two other studies (Plikaytis, et al., supra; Telenti, et al., supra) described similar PCR strategies for Mycobacterial speciation, but these methods required the detection of restriction enzyme site polymorphisms (RFLP) within the PCR amplified products. Both methods relied on the use of two restriction enzymes for differentiating the Mycobacterial species. Also, intraspecies DNA RFLPs were observed. In initial studies with clinical samples (Telenti, et al., supra), problems were observed. . .

SUMM . . . and classification of organisms. The identification of highly conserved regions flanking a variable region led to the production of universal primers which can be used to specifically amplify these variable regions of nucleic acid, thereby providing a target sequence for use. . .

SUMM The primers and the method of the invention are useful for the identification of organisms, including pathogens and non-pathogens, isolated from human/animal,. . .

DRWD . . . 3' (designated as H280) flanking region of HSP60 for over 40 different organisms, SEQ ID NOS:5-92, to which the oligonucleotide primers of the invention hybridize.

DETD The present invention provides oligonucleotide primer(s) for identification of a organism wherein the identification includes amplification of variable regions of a polynucleotide sequence encoding a heat. . .

DETD In a first embodiment, the present invention provides isolated oligonucleotide primer(s) for identification of an organism wherein the identification includes amplification of a polynucleotide sequence encoding a region of a heat. . .

DETD . . . B. subtilis, Streptococcus faecalis, Bartonella henselae, B. quintana, B. bacilliformis, Yersinia pseudotuberculosis, Vibrio cholera, Legionella pneumophila, Helicobacter pylori, Neisseria gonorrhoeae, Mycobacterium marinum, Candida albicans, and P. aeruginosa as well as organisms listed in FIGS. 1 and 4 herein.

DETD The identification of a species of organism is accomplished by oligonucleotide(s) which are primers for amplification of the highly conserved region of a genomic locus having the sequence of a heat shock protein. These unique oligonucleotide primers were produced based upon identification of the flanking regions contiguous with a region of the heat shock protein, HSP60, locus. These oligonucleotide primers comprise sequences which are capable of hybridizing with the flanking nucleotide sequence having substantially the sequence:

DETD . . . stringent conditions and sequences having sufficient homology with SEQ ID NO: 1 and SEQ ID NO:2, such that the oligonucleotide primers of the invention hybridize to the sequence.

DETD The primers of the invention embrace oligonucleotides of sufficient length and appropriate sequence so as to provide specific initiation of polymerization on a significant number of nucleic acids in the polymorphic locus. Specifically, the term "primer" as used herein refers to a sequence comprising two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and most. . . presence of nucleoside triphosphates and an agent for polymerization, such as DNA polymerase, and a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The oligonucleotide primer typically contains 12-20 or more nucleotides, although it may contain fewer nucleotides.

DETD Primers of the invention are designed to be "substantially" complementary to each strand of the genomic locus to be amplified. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions which allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with the 5' and 3' flanking sequences to hybridize therewith and permit amplification of the genomic. . . 5' and 3' flanking sequences for the genomic locus of over 40 different prokaryotic species which are amplified by the primers of the invention. Preferably, the primers of the invention include:

DETD where A is adenosine, T is thymidine, C is cytosine, G is guanosine and I is inosine. Primers having substantial homology to SEQ ID NO:3 and SEQ ID NO:4 are also included in the present invention.

DETD Oligonucleotide primers of the invention are employed in the amplification process which is an enzymatic chain reaction that produces exponential quantities of polymorphic locus relative to the number of reaction steps involved. Typically, one primer is complementary to the negative (-) strand of the polymorphic locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA Polymerase I (Klenow). . . . - strands containing the target polymorphic locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension results in exponential production of the region (i.e., the target polymorphic locus sequence) defined by the primer. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

DETD The oligonucleotide primers of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments. . .

DETD . . . may also be employed, or the nucleic acids produced in a previous amplification reaction herein, using the same or different primers may be so utilized. The specific nucleic acid sequence to be amplified, i.e., the polymorphic locus, may be a fraction. . .

DETD . . . as the template. Strand separation can be effected either as a separate step or simultaneously with the synthesis of the primer extension products. This strand separation can be accomplished using various suitable denaturing conditions, including physical, chemical, or enzymatic means, the. . .

DETD . . . acid containing the sequence to be amplified is single stranded, its complement is synthesized by adding one or two oligonucleotide primers. If a single primer is utilized, a primer extension product is synthesized in the presence of primer, an agent for polymerization, and the four nucleoside triphosphates described below. The product will be partially complementary to the single-stranded. . . unequal length strands that may then be separated into single strands to produce two single separated complementary strands. Alternatively, two primers may be added to the single-stranded nucleic acid and the reaction carried out as described.

DETD . . . as a template for the synthesis of additional nucleic acid strands. This synthesis is performed under conditions allowing hybridization of primers to templates to occur. Generally synthesis occurs in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. Preferably, a molar excess (for genomic nucleic acid, usually about 10.sup.8 :1 primer:template) of the two oligonucleotide primers is added to the buffer containing the separated template strands. It is understood, however, that the mount of complementary strand. . . may not be known if the process of the invention is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter,

however, the amount of primer added will generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified. . .

DETD The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90°-100° C. from about 1 to 10 minutes, preferably. . . 4 minutes. After this heating period, the solution is allowed to cool to room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art.. . .

DETD The agent for polymerization may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes.

DETD . . . polymerase, other available DNA polymerases, polymerase muteins, reverse transcriptase, and other enzymes, including heat-stable enzymes (i.e., those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation). Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each polymorphic locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may. . .

DETD The above process is repeated on the single-stranded molecules. Additional agent for polymerization, nucleotides, and primers may be added, if necessary, for the reaction to proceed under the conditions prescribed above. Again, the synthesis will be initiated at one end of each of the oligonucleotide primers and will proceed along the single strands of the template to produce additional nucleic acid. After this step, half of the extension product will consist of the specific nucleic acid sequence bounded by the two primers.

DETD Now that the present invention has provided novel oligonucleotide primers for the amplification of a variable genomic region, the invention provides a method for the identification of the species of an organism comprising amplifying a region of the genomic nucleic acid of the organism by means of oligonucleotide primers which hybridize to target flanking 5' and 3' polynucleotide sequences of the genomic nucleic acid, the target polynucleotide sequence having. . .

DETD . . . amplification have been described and can also be employed as long as the HSP60 locus amplified by PCR using the primers of the invention is similarly amplified by the alternative means. Such alternative amplification systems include but are not limited to. . . acid amplification technique is nucleic acid sequence-based amplification (NASBA) which uses reverse transcription and T7 RNA polymerase and incorporates two primers to target its cycling scheme. NASBA can begin with either DNA or RNA and finish with either, and amplifies to. . . Alternatively, nucleic acid can be amplified by ligation activated transcription (LAT). LAT works from a single-stranded template with a single primer that is partially single-stranded and partially double-stranded. Amplification is initiated by ligating a cDNA to the promoter oligonucleotide and within. . . fills and joins the gap, mimicking normal DNA repair. Nucleic acid amplification by strand displacement activation (SDA) utilizes a short primer containing a recognition site for HincII with a short overhang on the 5' end which binds to target DNA. A DNA polymerase fills in the part of the primer opposite the overhang with sulfur-containing adenine analogs. HincII is added but only cut the unmodified DNA strand. A DNA polymerase that lacks 5' exonuclease activity enters at the site of the nick and begins to polymerize,

displacing the initial primer strand downstream and building a new one which serves as more primer. SDA produces greater than 10.sup.7 -fold amplification in 2 hours at 37° C. Unlike PCR and LCR, SDA does not. . .

DETD Another embodiment of the invention provides a target genomic polynucleotide locus which is defined by being amplified by the primers of the invention identified by SEQ ID NO:3 and SEQ ID NO:4, or primer sequences substantially complementary thereto, wherein the polynucleotide locus does not hybridize with a polynucleotide locus from Staphylococcus aureus amplified by primers of the invention identified by SEQ ID NO:3 and SEQ ID NO:4, or primer sequences substantially complementary thereto. The genomic locus defined by amplification of the primers as described herein encodes a heat shock polypeptide, e.g., HSP60.

DETD . . . one of the container means may comprise means for amplifying target DNA, said means comprising the necessary enzyme(s) and oligonucleotide primers for amplifying said target DNA from the organism or a cell of the organism. The oligonucleotide primers include primers having a sequence:

DETD or primer sequences substantially complementary thereto. The target flanking 5' and 3' polynucleotide sequence has substantially the sequence selected from the group. . .

DETD . . . 50 ng of genomic DNA, 2 U of Taq DNA polymerase (GIBCO) and 0.5 μg of each of the degenerate primers H279 (SEQ ID NO:3) and H280 (SEQ ID NO:4). A final volume made up to 100 μl with dH.sub.2 O. . .

DETD The sequences of the two primers were

DETD for H279 and H280, respectively. Inosine (I) was used to reduce the degeneracy of the primers. The last 26 nucleic acid residues of primers H279 and H280 correspond to DNA residue numbers 688 to 713 and the complement of residue numbers 1267 to 1292,. . .

DETD . . . protocols. Labelling of the 600 bp fragments for use as probes was carried out using Digoxigenin-11-dUTP and the standard random primer method (Maniatis, et al., supra, Molecular Cloning: A Laboratory Manual; Boehringer Mannheim protocols).

DETD FIG. 2 shows the results of experiments used to determine (i) if the degenerate primers could be used to amplify specific Staphylococcal targets from mixed cultures and (ii) if species specific HSP 60 probes identify. . .

DETD The degenerate primers of the invention were capable of amplifying a 600 bp putative HSP 60 fragment from all Staphylococci species and subspecies listed in FIG. 4. Thus, species specific probes can be easily generated with such primers. The Examples presented herein concentrated specifically on six species responsible for the majority of clinical Staphylococcal infections (Kloos and Lamba,. . .

DETD . . . targeted organisms in clinical samples, especially those from normally sterile sites such as cerebrospinal fluid and sera. The degenerate PCR primers have been used successfully as illustrated herein, to amplify the expected 600 bp HSP 60 fragment from diverse organisms. Though. . .

CLM What is claimed is:

1. A genomic polynucleotide locus which is defined by being amplified by primers having a sequence: 5'-GAIIIIGCIGGIGA(T/C)GGIACIACIAC-3' (SEQ. ID NO: 3) and 5'-(T/C)(T/G)I(T/C)(T/G)ITCICC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ID NO:4) wherein the amplified polynucleotide locus from one species does not hybridize under high stringency conditions with a polynucleotide locus from another species amplified by primers having a sequence: 5'-GAIIIIGCIGGIGA(T/C)GGIACIACIAC-3' (SEQ ID NO:3) and 5'-(T/C)(T/G)I(T/C)(T/G)ITCICC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ID NO:4). 2.

3. Isolated oligonucleotide primer(s) for use in the identification of the species of an organism wherein the primer

hybridizes with a target polynucleotide sequence consisting of the sequence selected from the group consisting of: 5'-GTTGTCGTACC(G/A)TCACCAGCAATTTC-3' (SEQ. ID NO:1),. . . 5'-AA(G/A)GCGCCTGGTTT(C/T)GGTGAT(C/A)(G/A)(A/T/C/G) (C/A)(G/A)-3' (SEQ. ID NO:2), 5'-GTIGTIGTICC(A/G)TCICCIGCIIIITC-3' (SEQ ID NO:93), and 5'-AA(A/G)GCICCIGGITT(T/C)GGIGAI(A/C)(A/C)I(A/C)(A/G)-3' (SEQ ID NO:94), and sequences complementary thereto wherein the primers amplify the genetic locus of claim 1.

4. The primer of claim 3, wherein the primer is 5'-GAIIIIGCIGGIGA(T/C)GGIACIACIAC-3' (SEQ ID NO:3) or 5'-(T/C)(T/G)I(T/C)(T/G)ITCICC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ID NO:4). 5.

5. The primer of claim 3, wherein the organism is a microorganism.

6. The primer of claim 5, wherein the organism is a prokaryote.

7. The primer of claim 6, wherein the prokaryote is a member of a genus selected from the group consisting of Staphylococcus, Pseudomonas,. . .
8. The primer of claim 7, wherein the species of the genus is selected from the group consisting of S. haemolyticus, S. epidermidis,. . .
. . . species of an organism, the kit comprising means for amplifying target DNA, said means comprising the necessary enzyme(s) and oligonucleotide primers for amplifying said target DNA from the organism or a cell of the organisms said primers consisting of a sequence: 5'-GAIIIIGCIGGIGA(T/C)GGIACIACIAC-3' (SEQ ID NO:3) and 5'-(T/C)(T/G)I(T/C)(T/G)ITCCC(A/G)AAICCIGGIGC(T/C)TT-3' (SEQ ID NO:4). 10.